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Evaluation of the Tooth-wear and Replacement Method for Aging White-tailed Deer (*Odocoileus virginianus*) on the Blue Grass Army Depot, Madison County, Kentucky

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ABSTRACT

We evaluated the reliability of the tooth-wear and replacement method for aging hunter-harvested deer on the Blue Grass Army Depot, Madison County, Kentucky. Eighty-seven hunter-harvested white-tailed deer were aged using the tooth-wear and replacement procedure and had an incisor tooth removed for aging via the cementum annuli method. We found no significant difference ($P < 0.05$) in the distribution of ages (1.5 years, 2.5 years, 3.5 years, ≥4.5 years) assigned using either method for male ($X^2 = 6.69$) or female ($X^2 = 6.85$) deer. The majority (77%) of the tooth-wear: cementum pairs of age estimates were in agreement for deer in this study. The tooth-wear and replacement technique tended to identify the age of female white-tailed deer ≥3.5 years of age incorrectly.
KEY WORDS: tooth-wear aging method, white-tailed deer, *Odocoileus virginianus*

INTRODUCTION

To assure hunter harvest does not become an additive form of mortality in white-tailed deer (*Odocoileus virginianus*) populations, resource managers need to know the age distribution of the harvest (Roseberry and Woolf 1991). Various methods have been used to determine the age of deer; e.g., antler beam diameter (Lueth 1963), body mass (Lueth 1963), eye lens weight (Connolly et al. 1969), incisor wear (Main and Owens 1995), hoof characteristics (Haugen and Speake 1958), molar tooth ratios (Robinette et al. 1957), mandibular tooth row length (Governo et al. 2006), cementum annuli (Asmus and Weckerly 2011), dentine:enamel ratios (Meares et al. 2006) and tooth-wear and replacement [the "Severinghaus Method," Severinghaus (1949)]. Although a regression formula depicting the relationship of eye lens weight to the age of central Kentucky white-tailed was developed by Keller and Landry (1976), the most common aging procedure used by resource agencies in the region is the tooth-wear and replacement method.

The accuracy of the tooth-wear and replacement procedure has been assessed in a number of states [see review by Gee et al. (2002)], but investigations centered in the Southeast are limited [e.g., Mississippi (Hackett et al. 1979; Jacobson and Reiner 1989; Mitchell and Smith 1991)]. Patterns of tooth-wear can vary between sexes of white-tailed deer (Van Deelen et al. 2000) and as the result of regional or local differences in soils and diet (Dimmick and Pelton 1994). Van Deelen et al. (2000) recommended that tooth-wear and replacement aging, when used in managing a specific white-tailed deer population, should be calibrated with local known-age specimens of both sexes. To our knowledge, an evaluation of the tooth-wear and replacement method for aging white-tailed deer in Kentucky has not been performed. The purpose of this study was to determine the reliability of the tooth-wear and replacement method for aging hunter-harvested deer on the Blue Grass Army Depot, Madison County, Kentucky.

MATERIALS AND METHODS

The Blue Grass Army Depot (BGAD) is a 5900 ha tract of land located 11 km south-southeast of Richmond, Kentucky. The Depot is primarily involved in activities associated

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Table 1. Summary of age determination for 87 white-tailed deer (*Odocoileus virginianus*) on the Blue Grass Army Depot, Madison County, Kentucky. The age of each animal was determined using two techniques; the tooth-wear and replacement procedure (Toothwear) and the cementum annuli (Cementum) method. Sample size is in parentheses.

MALES		FEMALES	
Toothwear	Cementum	Toothwear	Cementum
1.5 yrs (34)	1 yr (26)	1.5 yrs (11)	1 yr (7)
2.5 yrs (28)	2 yrs (26)	2.5 yrs (5)	2 yrs (5)
3.5 yrs (5)	3 yrs (12	3.5 yrs (2)	3 yrs (1)
≥4.5 yrs (0)	4 yrs (3)	≥4.5 yrs (2)	4 yrs (1)
			6 yrs (1)
		7 yrs (2)	
		9 yrs (1)	
			10 yrs (1)
		13 yrs (1)	

with the storage and maintenance of conventional and chemical munitions. As part of the Depot's management of natural resources, white-tailed deer hunts are typically held each year. Because of security concerns, the hunt is highly regulated and all successful hunters are required to bring deer to a check station to be weighed and aged. Aging is performed by a Kentucky Department of Fish and Wildlife Resources (KDFWR) biologist who has experience aging deer using the tooth wear and replacement method. During 1989 hunts, adult deer (no fawns) brought to the Depot check station were randomly selected for use in this study. Each deer was aged by the same KDFWR biologist using the tooth-wear and replacement method. Animals were assigned to an age category (1.5 years, 2.5 years, 3.5 years, ≥4.5 years) and an incisor tooth removed. Incisors were aged (Matson's Laboratory, Milltown, MT) using the cementum analysis technique (Dimmick and Pelton 1994).

In a review of published literature, Asmus and Weckerly (2011) noted sectioned teeth of white-tailed deer from populations in the southeastern United States have been reported to contain cementum annuli that were difficult to interpret because the annuli often merged together and were poorly defined. Asmus and Weckerly (2011) found the precision of the cementum analysis procedure varied among years and between sexes of mule deer (*O. hemionus*) from the same population. However, other researchers have reported the cementum analysis procedure to be more accurate than the tooth-wear and replacement technique for aging deer (Sauer 1971; Hamlin et al. 2000; Gee et al. 2002); we assumed that the ages determined by cementum analysis for deer analyzed in this study represented the animal's true age.

RESULTS

Certainty codes supplied by Matson's Laboratory indicated the amount of confidence in the accuracy of each cementum age estimate. The codes are defined as: (A) "Some tooth sections have a distinct annulus pattern and the result of age analysis is nearly certain;" (B) "There is histological evidence to support the reported cementum age. If error is present, it would be likely within the range given;" and (C) "There is little histological evidence to support the reported cementum age, which might be within the range given." Of the 105 white-tailed deer incisors submitted, 15 were assigned certainty code B and 3 to code C; these were deleted from the analysis. Of the 87 deer teeth utilized in this study, 77% (n = 67) were from males; 23% (n = 20) from females (Table 1). There was no significant difference ($P < 0.05$) in the distribution of ages (1.5 years, 2.5 years, 3.5 years, ≥4.5 years) assigned using either age determination method for male ($X^2 = 6.69$) or female ($X^2 = 6.85$) deer. For the chi square analysis the following procedure was employed, animals in a particular age category determined via the tooth-wear procedure (e.g., 1.5 years) were the 'observed' value; animals assigned an age via the cementum annuli procedure (e.g., 1 year) were considered the 'expected' value (all female deer identified as 4 years or older by the cementum annuli procedure were combined to form the 'expected' category). Accepting an age spread of 6 months, 77% of the tooth-wear: cementum pairs of age estimates were in agreement for white-tailed deer in this study (e.g., a deer assigned a tooth-wear age of 1.5 years and cementum annuli age of 2 years was considered in agreement, tooth-wear age of 2.5 years and cementum annuli age of 3 years was considered in agreement, etc.). Of the 20 tooth-wear: cementum pairs of age estimates which varied by more than 6 months, the majority of the aging disagreements (70%; 6 males, 8

females) involved the tooth-wear assigned age being lower than the cementum annuli assigned age by an average of 1.1 years for males; 4.0 years for females.

DISCUSSION

This study was site-specific, samples were obtained from deer in the same population, and tooth-wear ageing was performed by the same individual. As noted by Gee et al. (2002) and Van Deelen (2000), these conditions should have minimized many of the variables (i.e., soil type habitat quality, range condition, variation between multiple individuals aging deer) that theoretically affect the accuracy of the tooth-wear and replacement aging technique.

Using jaw bones from 98 known-aged white-tailed deer, Jacobson and Reiner (1989) had 55 deer biologists from southeastern states age the jaws using the tooth-wear and replacement method. The biologists correctly aged 96%, 87%, and 77% of the mandibles from fawns, yearlings, and 2.5 year old deer, respectively, but were correct only 25% of the time for animals ≥3.5 years old (Jacobson and Reiner 1989). The tendency to incorrectly identify the age of white-tailed deer ≥3.5 years of age using the tooth-wear and replacement technique was evident in this study (especially in female deer) and has been reported by biologists from various states, e.g., Michigan (Ryel et al. 1961), Maine (Gilbert and Stolt 1970), Texas (Cook and Hart 1979), Montana (Hamlin et al. 2000), Oklahoma (Gee et al. 2002).

Gee et al. (2002) reported that 34 white-tailed deer biologists from various southeastern states, when given 106 jaw bones or dental casts from known-aged deer, failed 60% of the time to correctly age deer ≥2 years old using the tooth-wear and replacement method. Gee et al. (2002) concluded that using the tooth-wear aging technique to determine ages of white-tailed deer beyond the general categories of fawn, yearling, and adult are unfounded; and indicated a review of published data sets (i.e., 6 articles) using known-age deer supported their conclusion.

In evaluating the accuracy of ages obtained for white-tailed deer, mule deer, and elk (*Cervus elaphus*) using the tooth-wear and replacement method and cementum analysis of incisors, Hamlin et al. (2000) reported the inaccuracy of ages assigned by the tooth-wear criteria, and the lack of compensation for the inaccuracy, are important considerations for even relatively simple tests of management practices. Nevertheless, they also noted the extra expense of obtaining age by the cementum annuli method would not be warranted if a management question concerned only fawn, yearlings, 2-year old, and ≥3 year old age categories (Hamlin et al. 2000). Current aging protocol on the Blue Grass Army Depot requires reporting the age of harvested deer using the tooth-wear and replacement method; but only within age categories of ≤6 months, 1.5 years, 2.5 years, and ≥3 years. Given the results of this study, we recommend the BGAD continue to use their current four category tooth-wear aging method; in which the maximum age assigned to a harvested deer is ≥3 years. However, we concur with Hamlin et al. (2000) who noted that should management needs make it necessary to determine whether specific physical or population parameters change significantly with the age of deer in a population (like the deer herd on the BGAD); harvested animals should be aged using the cementum annuli method.

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Occurrence of Three Leech Species (Annelida: Hirudinida) on Fishes in the Kentucky River

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ABSTRACT

Leeches were collected from six fish species distributed among four of ten sites sampled. The leech species observed were *Myzobdella reducta* (Meyer 1940) and *Myzobdella lugubris* Leidy, 1851 of the family Piscicolidae and *Placobdella pediculata* Hemingway, 1908 of the family Glossiphoniidae. Attachment locations for *Myzobdella lugubris* included various sites in the buccal cavity of green sunfish [*Lepomis cyanellus* (Rafinesque)], largemouth bass [*Micropterus salmoides* (Lacepède)], and spotted bass [*Micropterus punctulatus* (Rafinesque)] and posterior to the eye of a largemouth bass. Attachment locations for *Myzobdella reducta* included on the caudal and pelvic fins of logperch [*Percina caprodes* (Rafinesque)] and the caudal and anal fin of spotfin shiners [*Cyprinella spiloptera* (Cope)] – a new host record. Attachment location observed for *Placobdella pediculata* was on the inside of the operculum of a freshwater drum [*Aplodinotus grunniens* (Rafinesque)]. This study established new host and attachment locations, the diverse presence of *Myzobdella reducta* on various fish species, and substantiated the high degree of host specificity of *Placobdella pediculata* for the freshwater drum.

KEY WORDS: Hirudinida, host fish, attachment locations, new host record, Kentucky River

INTRODUCTION

Recent phylogenetic analyses have greatly enhanced our knowledge of the evolutionary relationships among leeches (Siddall et al. 2005; Williams and Burreson 2006). However, remaining limitations in distributional and life history data hamper our capacity for a more complete understanding, with substantial gaps in knowledge for the Commonwealth of Kentucky. Published accounts have documented the host relationships for a few leech species in a limited geographic area (White and Crisp 1973; White 1974; Bauer and Branson 1975; Klemm 1985). During the course of a fish study by one of the authors (J. E. F.), specimens were examined for attached leeches and resulted in several new host relationships documented in this paper.

MATERIALS AND METHODS

In the summer of 2000, electrofishing was conducted at 10 sites on the mainstem of the Kentucky River, Kentucky (Table 1; Figure 1). Electrofishing was conducted following the methods outlined in (Lazorchak et al. 2000), with the modification that 1000 m was electrofished on each bank of the river. Electrofishing was conducted in a downstream direction along the main-channel riparian habitat of each bank at a speed near or slightly exceeding the flow rate of the system if flow rates were low (Lazorchak et al. 2000; Ohio EPA 1989; Reynolds 1983). All fish were identified, examined for leeches, and then released, with the exception of representative vouchers specimens retained for laboratory identification. Voucher specimens were fixed and preserved with formaldehyde in the field and transported to the laboratory. During laboratory identification (principal keys: Etnier and Starnes 1993; Pflieger 1975; Stauffer et al. 1995; Trautman 1981), each fish specimen was carefully examined for leeches.

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Table 1. Latitude and longitude of sites sampled on the Kentucky River.

River	River kilometer	Site ID	Longitude	Latitude
Kentucky 01	37.75	K01	−84.956850	38.465090
Kentucky 02	83.54	K02	−84.861230	38.317230
Kentucky 03	124.63	K03	−84.838510	38.096980
Kentucky 04	168.95	K04	−84.764900	37.881470
Kentucky 05	215.32	K05	−84.638560	37.774780
Kentucky 06	251.00	K06	−84.459340	37.840450
Kentucky 07	280.77	K07	−84.273170	37.914090
Kentucky 08	305.19	K08	−84.100150	37.858530
Kentucky 09	348.75	K09	−83.993850	37.709190
Kentucky 10	407.88	K10	−83.724100	37.567370

Leeches were relaxed in 5% ethanol (added dropwise in a vessel until the leech no longer reacted to a probe), fixed in 10% buffered formalin, preserved in 70% ethanol, and examined under a dissecting microscope. Voucher specimens of leeches were deposited in the Invertebrate Zoology collections of the National Museum of Natural History, Smithsonian Institution (accession no. 2057347).

RESULTS

A total of 3593 fish were collected during sampling; the majority of which were identified and released live in the field. During field

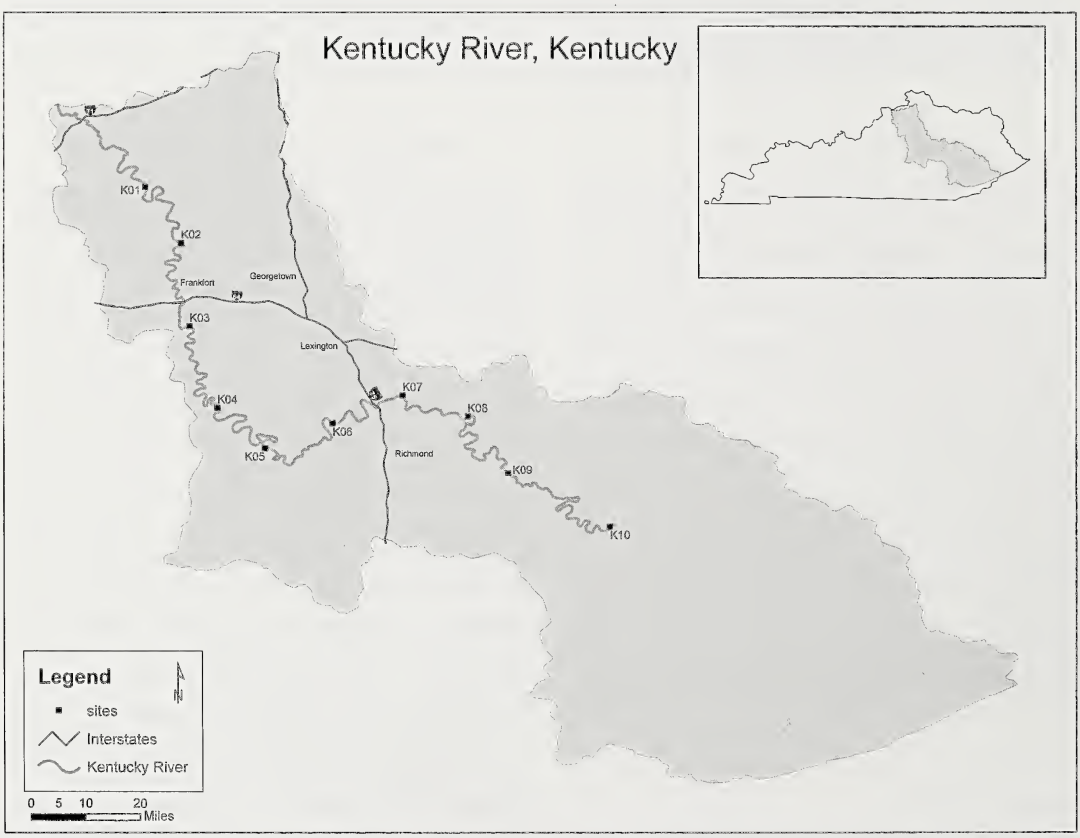

Figure 1. Collection sites on the main stem of the Kentucky River, identified by site ID.

Figure 2. *Actinobdella pediculata* attached to the inside surface of the operculum of a freshwater drum (*Aplodinotus grunniens*).

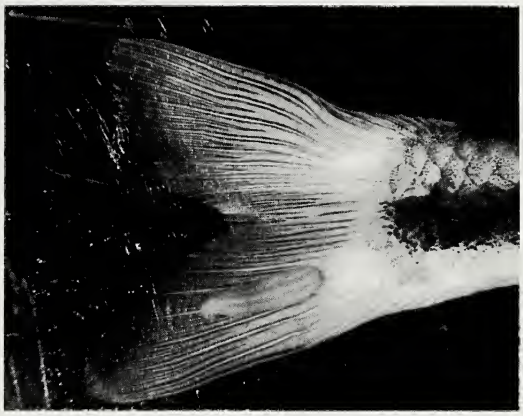

Figure 4. *Piscicolaria reducta* attached to the caudal fin of a spotfin shiner (*Notropis spilopterus*).

processing, *Placobdella pediculata* Hemingway, 1908 was collected, and during laboratory identification of vouchered specimens, two additional leech species were collected – *Myzobdella lugubris* Leidy, 1851 and *Myzobdella reducta* (Meyer 1940).

Placobdella pediculata was collected from inside the operculum on the last gill arch of a freshwater drum [*Aplodinotus grunniens* (Rafinesque)] at site K01 (Figure 2). *Placobdella pediculata* is not commonly encountered and found in the Great Lake and Mississippi River drainage systems (Klemm 1982, 1985). This leech species is host-specific to *A. grunniens*, where it permanently attaches inside the operculum area (Klemm 1985; Bur 1994).

Figure 3. *Myzobdella lugubris* attached inside the buccal cavity of a of a largemouth bass (*Micropterus salmoides*).

Specimens of *Myzobdella lugubris* were collected from the buccal cavity of green sunfish [*Lepomis cyanellus* (Rafinesque)] and juvenile largemouth bass [*Micropterus salmoides* (Lacepède)] from site K02 and juvenile spotted bass [*Micropterus punctulatus* (Rafinesque)] from site K05 (Figure 3). Those of the spotted bass were consistently attached to the roof of the buccal cavity and difficult to detect. An additional specimen was collected posterior to the eye of a largemouth bass from site K03. *Myzobdella lugubris* is a common and widely-distributed opportunistic blood-feeding leech on fish (Klemm 1982, 1985). Any fish species occurring in North America is considered a potential host.

Myzobdella reducta was collected from the caudal and anal fins of spotfin shiners (Figure 4) [*Cyprinella spiloptera* (Cope)] at sites K03 and K05, respectively, and on the caudal and pectoral fins of a logperch [*Percina caprodes* (Rafinesque)] at site K05. The spotfin shiner (*Cyprinella spiloptera*) is a new host record for *Myzobdella reducta*. *Myzobdella reducta* is infrequently collected and has a scattered distribution in eastern North America (Klemm 1982, 1985). It is also an opportunistic blood-feeding leech on fish. The reported hosts of *Myzobdella reducta* are listed in Table 2.

DISCUSSION

Leeches are common in freshwaters and generally more common in lentic than in lotic systems. As part of their normal life cycles,

Table 2. Reported host fish of *Myzobdella reducta* (Meyer 1940).

Fish species	Common name	References
Ameiurus melas (Rafinesque)	black bullhead	Harms 1959, 1960
Erimyzon sucetta (Lacepède)	lake chubsucker	Price and Nadolny 1993
Etheostoma blennioides Rafinesque	greenside darter	Bauer and Branson 1975; Bauer 1976; Murray et al. 1977
Etheostoma caeruleum Storer	rainbow darter	Bauer and Branson 1975; Erickson 1976; Kozel and Whittaker 1982
Etheostoma sagitta (Jordan and Swain)	arrow darter	Klemm 1982
Etheostoma stigmaeum (Jordan)	speckled darter	Bauer and Branson 1975
Etheostoma virgatum (Jordan)	striped darter	Bauer and Branson 1975
Etheostoma zonale (Cope)	banded darter	Bauer and Branson 1975; Erickson 1976, 1978
Ictalurus punctatus (Rafinesque)	channel catfish	Harms 1959, 1960; Nagel 1976; Wetzel 1982
Lepomis auritus (Linnaeus)	redbreast sunfish	Price and Nadolny 1993
Lepomis cyanellus Rafinesque	green sunfish	Klemm 1972
Chaenobryttus gulosus (Cuvier in Cuvier and Valenciennes)	warmouth	Price and Nadolny 1993
Lepomis macrochirus Rafinesque	bluegill	Meyer 1946; Petty and Magnuson 1974
Lepomis punctatus (Valenciennes in Cuvier and Valenciennes)	spotted sunfish	Booth and Aliff 1978; Klemm 1985; Price and Nadolny 1993
Notemigonus crysoleucas (Mitchill)	golden shiner	Meyer 1954
Notropis atherinoides Rafinesque	emerald shiner	White and Crisp 1973
Cyprinella spiloptera (Cope)	spotfin shiner	This study
Percina aurantiaca (Cope)	tangerine darter	Bauer 1976
Percina caprodes (Rafinesque)	logperch	White and Crisp 1973; Bauer and Branson 1975; Bauer 1976; White 1977; Schramm et al. 1981; Appy and Cone 1982; This study
Percina copelandi (Jordan)	channel darter	Bauer 1976
Percina evides (Jordan and Copeland in Jordan)	gilt darter	Bauer and Branson 1975; Bauer 1976; Erickson 1976
Percina maculata (Girard)	blackside darter	Bauer and Branson 1975; Bauer 1976; Erickson 1976
Percina phoxocephala (Nelson)	slenderhead darter	Meyer 1940, 1946; Page and Smith 1971; Erickson 1976
Percina sciera (Swain)	dusky darter	Page and Smith 1971
Pomoxis nigromaculatus (Lesueur in Cuvier and Valenciennes)	black crappie	Price and Nadolny 1993
Tilapia aurea (Steindachner)	blue tilapia	Price and Nadolny 1993

many leeches parasitize a variety of hosts and feed on blood and body fluids. In doing so, they deprive their hosts of important nutrients (Sawyer, 1986). When an overabundance of leeches occurs in a system, it is generally considered to be an indicator of poor water quality, especially in lotic systems (McDonald et al. 1990).

Fish leeches (Glossiphonidae and Piscicolidae) commonly attach to various sites on the body of the host, including the pectoral, pelvic, dorsal, and caudal fins; the eyes; the interior of the gill chamber; the inside of the mouth cavity; and directly to the main body of the fish (as reviewed by Schulz et al. 2011). The presence of leeches in high numbers can negatively impact the recreational value of an aquatic system to local communities because people generally find them undesirable and view their presence on and in fish as an indicator of poor health. Opportunities to document the presence and extent of leeches on fish in freshwater systems are therefore warranted.

Data presented in this study were derived from rapid examination of fish in the field supplemented with data collected during more thorough laboratory examination of vouchered fish specimens. As a result of this non-random study design, no estimates on the percentage of fish with leeches can be made from these data. Furthermore, even if it was possible to make these estimates, little is known about the expected occurrence rates of leeches in this system and it is, therefore, impossible to use these data to assess the condition of the Kentucky River.

From a life history perspective, this study established new host and attachment locations, the diverse presence of *Myzobdella reducta* on various fish species, and substantiated the high degree of host specificity of

Placobdella pediculata for the freshwater drum. Given the paucity of work on the distribution and life histories of leeches in Kentucky, it is probable that additional research in the Kentucky River Basin and other basins in the Commonwealth of Kentucky would produce new host and distribution records. As these records increase, the value of these organisms to serve as indicators of system condition will likewise increase.

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Size Structure of *Fagus grandifolia*, *Liriodendron tulipifera*, and *Celtis occidentalis* Populations in a Wetland Forest in Campbell County, Kentucky

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ABSTRACT

Size structure of a tree population not only reflects its past history but also how it is likely to change in the future. Three species, all located in a wetland forest in northern Kentucky near the Ohio River, were examined. This forest is part of a network of newly established permanent plots in North America, and the acquisition of baseline data for this network is critical. The *Fagus grandifolia* (American beech) population was the dominant species in a mature stand of approximately 1 ha, while the *Liriodendron tulipifera* (tulip-poplar) and *Celtis occidentalis* (hackberry) populations were located in an adjacent second-growth stand (also ca. 1 ha) that has developed over the past 50 years. *F. grandifolia* had a close fit to a power law function, but the deviations from the function suggest that the population is not currently reproducing fast enough to replace itself. *L. tulipifera*, on the other hand, had a bell-shaped size distribution, indicating that it is no longer reproducing and will decrease in importance over time. *C. occidentalis* had a very close fit to a power law function, suggesting it will maintain itself at current levels in the future. Current *F. grandifolia* levels in the younger secondary stand suggest that it will increase its importance in the future and will come to resemble the population in the mature stand.

KEY WORDS: American beech, tulip poplar, hackberry, diameter class, Campbell County, size structure

INTRODUCTION

The age structure of a plant population is not only a reflection of the history of the population but also indicates how the population is likely to change in the future, barring extreme stochastic events (Gurevitch et al. 2006). For many plants, especially perennials, age is either difficult to measure or is less important than size, or both. This is especially important for trees, since size is much better correlated with survivorship and reproduction than actual age. Although trees can be aged with an increment borer, diameter is much easier to measure, and so size structure is more frequently reported.

Age or size (diameter) distribution of trees in stands is often used to tell how the stand originated. Even-aged stands usually show a bell-shaped curve of number of trees vs. diameter class, sometimes with a long left tail (log-normal distribution; Smith et al. 1997). The trees in these stands all germinated or were released at about the same time. Trees that exhibit this kind of pattern are often shade-intolerant; they require some kind of disturbance to begin growth, but they don't regenerate under their own shade. Balanced uneven-aged stands, where regeneration is replacing mortality, show a reverse J-shaped (also called L-shaped) curve, with large numbers of small trees and gradually smaller numbers of larger trees (Smith et al. 1997). These kinds of trees are often shade-tolerant; they can regenerate under their own shade and thus can continue to regenerate as the stand develops. Many studies have found that *Fagus grandifolia* Ehrh. (American beech) often has this kind of distribution (Schmalzer 1988; Cho and Boerner 1991; Houle 1991; Busing 1998; Swanson and Vankat 2000; Woods 2000, 2004; Harcombe et al. 2002; Galbraith and Martin 2005; Ozier et al. 2006).

In stands with mixed species, size structure distributions must be used carefully. Different species can become established at different times after a forest is disturbed and a new stand is established. Although each population may be essentially even-aged, i.e., established at the same time, in aggregate the stand structure may have the reverse J-shape of a uneven-aged stand (e.g., Shotola et al. 1992; Lowenstein et al. 2000; Harcombe et al. 2002). And this stand structure may continue into the future, even though species composition changes. The history and possible future

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of each species can only be determined by examining them individually.

Leak (1975) showed that when the logarithm of number of trees in each age class is plotted against the logarithm of age, the shape of the resulting curves will indicate the state of the population. A straight line indicates a stationary or balanced uneven-age stand. A concave (reverse J- or L-shaped) curve indicates an increasing population, whereas a convex (bell-shaped) curve indicates a decreasing population that is no longer reproducing enough to replace itself.

While age and diameter increase together monotonically, they are not always related linearly. Lorimer (1980) found that age and diameter are related in *F. grandifolia* by a power law function, while a quadratic equation described *Liriodendron tulipifera* L. (tulip poplar). When a power law function holds, as for beech, log(age) is linearly proportional to log(diameter), but with a quadratic function, as for tulip poplar, log(age) is almost linearly proportional to untransformed diameter. When there is a linear relationship between age and diameter, as for *Celtis laevigata* Willd. (sugar hackberry; Barry and Kroll 1999), then the relationship between age and diameter is similar to that of a power law function [i.e., log(age) is proportional to log(diameter)]. Provided the age-diameter distribution of each species is understood, Leak's (1975) method can be applied to diameter data to determine the current structure of the population and how it is likely to fare over time as the stand develops.

The aim of this study was to determine the current size structure of three species found in a wetland forest in Campbell County, northern Kentucky: *F. grandifolia* (American beech), *L. tulipifera* (tulip-poplar), and *Celtis occidentialis* L. (hackberry). All three of these species have similar, widely-overlapping ranges in the eastern United States, but they vary considerably in their life histories and requirements. *Fagus grandifolia* is a relatively long-lived and slow-growing mesophytic species that is very shade-tolerant. It can grow on poorly-drained (but not flooded) soils, and it is usually found on acidic soils. It attains some of its best growth on alluvial soils of the Ohio River valley. *Liriodendron tulipifera* is shade-intolerant, but its fast growth and longevity allows it to persist in mature stands. It achieves its best growth on moderately moist, well-drained, and loose-textured soils and, like *F. grandifolia*, some of its best growth is found in the Ohio Valley. *Celtis occidentalis* is a small- to medium-size tree of only moderate longevity that is intermediate to tolerant for shade. It is widely tolerant of soil and moisture conditions; it is both drought-resistant and tolerant of occasional flooding. It achieves its best growth on valley soils (Burns and Honkala 1990). A number of population size structure descriptions have been published for *F. grandifolia* (Leak 1975; Schmalzer 1988; Cho and Boerner 1991; Woods 2004; Galbraith and Martin 2005) and *L. tulipifera* (Clebsch and Busing 1989; Busing 1998; Galbraith and Martin 2005; Ozier et al. 2006), but I am aware of only one for *C. occidentalis* (Swanson and Vankat 2000).

The forests of the southwest Ohio-southeast Indiana-northern Kentucky region have long been of interest because their location near three different forest types, including the *Fagus-Acer* forests to their north, *Quercus-Carya* forests to their west, and mixed mesophytic forests to their south and east (Braun 1950). Braun (1914, 1916) studied this area, which she called the Melbourne Forest. More recently, it was studied by Bryant (1987), Bryant and Held (2004), and Boyce et al. (2012). This wetland forest is now also part of the Ecological Research as Education Network Permanent Forest Plot Project (EREN PFPP; http://erenweb.org/project/carbon-storage-project/). Two 400-m^2 permanent plots were established in 2012. The goals of EREN PFPP are to examine long-term changes in forest ecosystems across the U.S. and Canada.

This study has three objectives: 1) determine what the current size structure indicates about the development of these forests; 2) determine how the these populations are likely to to change in the future, if the past and current conditions continue; and 3) establish baseline data against which to measure changes in size structure of these three contrasting species in the future.

MATERIALS AND METHODS

This study was carried out at the St. Anne Wetlands Research and Education Center (39° 2′ 4″ N, 84° 22′ 24″ W) in Melbourne,

Kentucky, U.S.A. This 100-ha wetland forest is located in Campbell County near the Ohio River and supports an extensive system of ephemeral ponds and streams. The study site is described in more detail in Boyce et al. (2012). Data for this study were collected in September–October 2011 from the same two adjacent sites (Mature Stand and Developing Stand; separated by a stream) described in that study. The approximate location and boundaries of the two sites are shown in Figure 1; each is ca. 1 ha in area. One of the EREN PFPP plots is located in the Mature Stand site, and the other is located in the Developing Stand area. Soils are silt loams in the Avonburg, Newark, Rossmoyne, and Wheeling series (Weisenberger et al. 1973).

Community structure was sampled with three parallel transects in each stand, each separated by 20 m. Along each transect, trees within 2.5 m of the transect were sampled for a distance of 40 m. Species and diameter at breast height (dbh) for trees with dbh ≥5 cm was determined. Density (ha^{-1}) and basal area ($m^2\ ha^{-1}$) were then calculated.

Population structure was determined by censusing each area for particular species. *F. grandifolia* was sampled from the Mature Stand only, while *L. tulipifera* and *C. occidentalis* were sampled only from the Developing Stand. Diameter was remeasured; in this census, individuals with dbh <5 cm were also tallied. Data were grouped into 10-cm size classes, e.g., >0–10 cm, >10–20 cm, etc., and the numbers for each size class were log-transformed. The midpoints of each size class were also log-transformed for *F. grandifolia* and *C. occidentalis*, but not for *L. tulipifera*, due to the different relations between size and age exhibited by these species. Data were then graphed and analyzed following the method of Leak (1975).

RESULTS & DISCUSSION

Although total basal area is similar for the two sites, density was much larger in in the Developing Stand, indicating that average tree size was smaller (Table 1). The Developing Stand also contained more species of trees. Within it, *Celtis occidentalis* had the greatest density, while *Liriodendron tulipifera* had the greatest basal area. Within the Mature Stand, *Fagus grandifolia* had the greatest density and the second largest basal area. While the two sites in this depression forest have similar elevations and are located next to each other, their histories are quite different. In 1949, the area of the Mature Stand had a completely closed canopy, while the area of the Developing Stand contained only a few scattered trees (Figure 1). The older age of the mature forest is reflected in the larger average tree size.

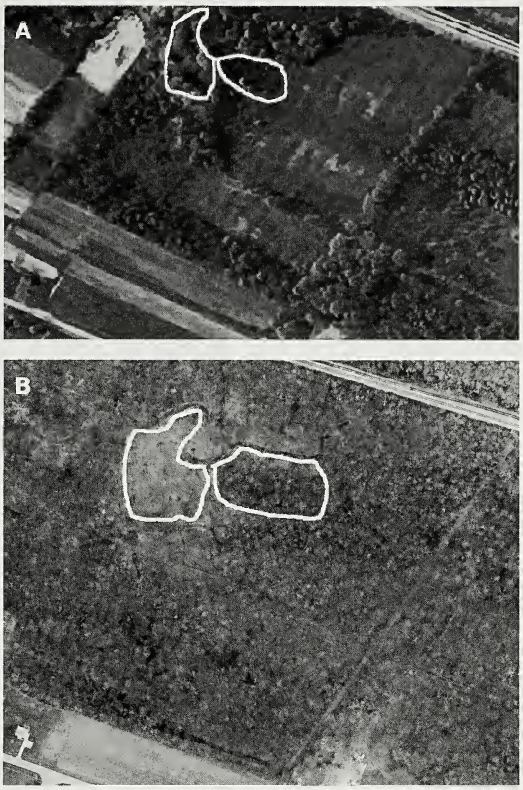

Figure 1. Approximate area of Mature Stand (on left) and Developing Stand (on right) in 1949 (A) and 2010 (B). Each stand is ~1 ha in size. Note that Mature Stand has complete canopy cover on both dates, while Developing Stand contains only scattered trees in 1949. The 1949 aerial photograph was obtained from USGS (2010), and the 2010 photograph was obtained from LINK-GIS (2012).

The *F. grandifolia* population in the Mature Stand shows declining numbers with increasing diameter class (Figure 2). While the fit between the power function (which appears as a straight line on a log-log plot) and the data is good ($R^2 = 0.85$), the data distribution is slightly concave. This indicates that while *F. grandifolia* is still reproducing, it is not doing so at a rate that will maintain its current

Table 1. Density and basal area of tree species in the Developing and Mature Stands. Data collected from trees with dbh ≥5 cm only.

Species	Developing stand		Mature stand	
	Density (ha^{-1})	Basal area ($m^2\ ha^{-1}$)	Density (ha^{-1})	Basal area ($m^2\ ha^{-1}$)
Acer negundo	16.7	0.58	–	–
Asimina triloba	33.3	0.11	–	–
Carya ovata	16.7	0.63	–	–
Celtis occidentalis	250.0	1.93	16.7	0.04
Cornus florida	16.7	0.06	–	–
Fagus grandifolia	116.7	1.70	150.0	14.20
Fraxinus americana	16.7	0.20	–	–
Liriodendron tulipifera	216.7	28.42	16.7	6.98
Prunus serotina	33.3	7.40	–	–
Quercus palustris	–	–	33.3	16.15
Sassafras albidum	–	–	33.3	0.22
Ulmus rubra	100.0	0.93	16.7	0.04
Totals	816.7	41.96	266.7	37.62

population (Leak 1975). A number of other studies have found similar trends for *F. grandifolia* (Leak 1975; Cho and Boerner 1991; Woods 2004; Galbraith and Martin 2005). This species is quite long-lived, however, and it has a much higher basal area and density than any other species in the Mature Stand, so it is likely to retain its dominance for some time.

Liriodendron tulipifera clearly has a bell-shaped population structure (Figure 3), indicating it is no longer reproducing. This is often seen in this species, at least in older stands (Clebsch and Busing 1989; Busing 1998; but see Schmalzer 1988; Galbraith and Martin 2005; Ozier et al. 2006). While this species is not currently reproducing, it is quite long-lived, and so it is expected to retain its dominance in the Developing Stand for some time. Successful regeneration could begin again if disturbance were to create gaps at of least 1.25–2.50 ha (Burns and Honkala 1990).

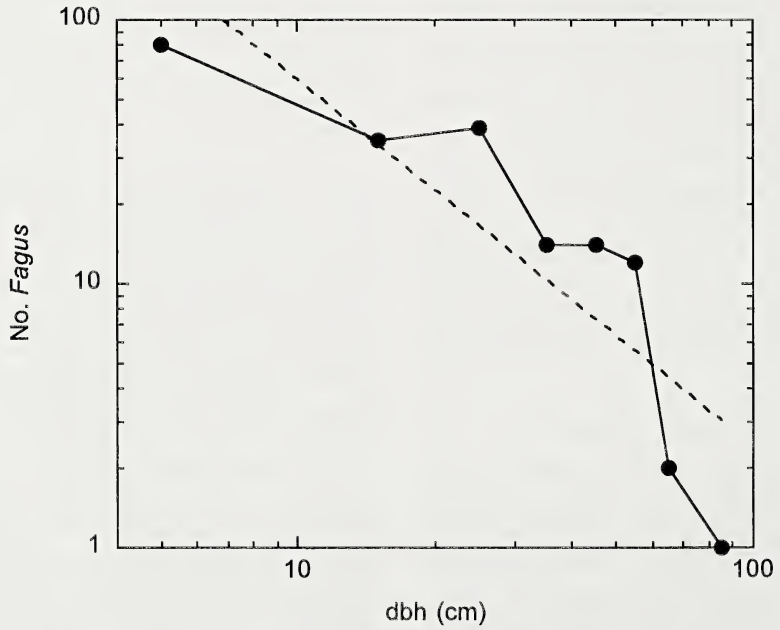

Figure 2. Population structure plot for *Fagus grandifolia* in the Mature Stand. Axes are on logarithmic scales; x-axis is dbh size-class mid point, and y-axis is number of trees in the size class. Data points are connected with solid line segments. The dotted line is a power function fitted to the data points: No. *Fagus* = $1447.4 \times^{-1.3866dbh}$, $R^2 = 0.85$.

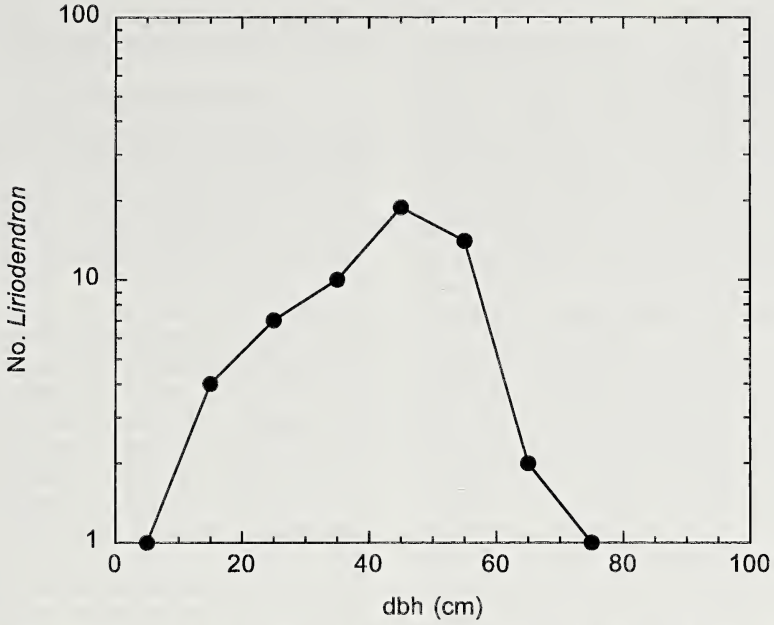

Figure 3. Population structure plot for *Liriodendron tulipifera* in the Developing Stand. Y-axis is on a logarithmic scale; x-axis is dbh size-class mid point, and y-axis is number of trees in the size class. Data points are connected with solid line segments.

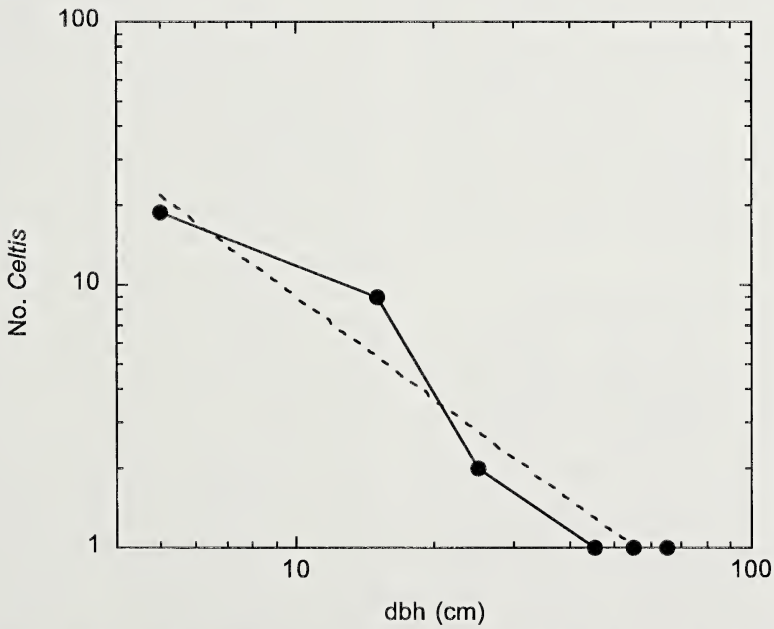

Figure 4. Population structure plot for *Celtis occidentalis* in the Developing Stand. Axes are on logarithmic scales; x-axis is dbh size-class mid point, and y-axis is number of trees in the size class. Data points are connected with solid line segments. The dotted line is a power function fitted to the data points: No. *Fagus* = $172.7 \times^{-1.2843 dbh}$, $R^2 = 0.94$.

Unlike *F. grandifolia* and *L. tuliperifa*, only one previous study on the population structure of *C. occidentalis* appears to have been published (Swanson and Vankat 2000), which showed a convex structure on a log-log scale, indicating an uneven-age stand that is increasing in importance. These same transformations were chosen for this study, based on the relationship between age and diameter of the closely related *C. laevigata* (Barry and Kroll 1999). The data show a very close fit to the power law function (R^2 = 0.94; Figure 4), indicating that *C. occidentalis* is replacing itself. This is consistent with previous work showing that it is has moderate to good shade tolerance (Burns and Honkala 1990). Unlike the other two species in this study, it is relatively short-lived. These findings suggest that, in the future, *C. occidentalis* is expected to retain a presence in the Developing Stand similar to that seen today. It is, however, much less important in the Mature Stand, so it is possible that shade cast by the dense canopy of *F. grandifolia*-dominated forest may inhibit the regeneration of *C. occidentalis*.

Fagus grandifolia basal area in the Developing Stand is currently low, but it has the third-highest density, after *C. occidentalis* and *L. tulipifera*. Over time and in the absence of large gap-creating disturbances, *F. grandifolia* is expected to increase its basal area in this stand and become dominant, as it currently is in the Mature Stand; a few large *L. tuliperifa* will remain, while *C. occidentalis* maintains a presence similar to or smaller than today. Thus, the Developing Stand is expected over the long term to become more like today's Mature Stand, at least in the absence of major gap-forming disturbances.

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Evaluation of Cultivars of Sunflower (*Helianthus annuus* L.) and Selected Environments for Production of Cut Flowers

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ABSTRACT

Sunflower *Helianthus annuus* is native to the United States and was domesticated by American Indians many centuries ago. Sunflowers exhibit wide variation and are grown worldwide for multiple purposes, including cut flowers. The present cut flower study involved 23 cultivars, three local environments, and performances data included in seed company catalogs, for cut flower production in Central Kentucky. Plant development data included seedling emergence, stem diameter, days to flowering, height at flowering, number of heads (marketable and nonmarketable) per plant, head diameter, vase life, and personal preference. There were evidences of both genetic and environmental variations for the plant characteristics. Flowering heads were rated as pleasing by florists and laypersons. The number of heads produced by some combinations of cultivars and environments would support profitable production. Further refinement is needed in the harvesting schedule and handling to reduce frequency of nonmarketable heads, to extend vase life, and to permit shipping greater distances. The results indicated that cut flowers have potential as a niche crop for Central Kentucky.

KEY WORDS: Sunflower, *Helianthus annuus*, cut flower, Western Kentucky Botanical Gardens, niche crop

INTRODUCTION

Sunflowers of the genus *Helianthus* are among the oldest, most widely grown, and taxonomically complex of all crops. More than 30 centuries ago, Indians in Southwest United States domesticated the native sunflower. Sunflower culture spread throughout the Indian Tribes of North America where the plants were widely used for food and nonfood purposes. Seeds were cracked and eaten like nuts or were ground and separated into flour and oil. Nonfood uses included dyes, medicines, textiles, and construction materials. Early Spanish explorers introduced sunflowers into Europe where they spread into Russia reaching their zenith as a crop. In the latter part of the 19th century, the sunflower was reintroduced into the United States for its recognized value as a silage crop and potential source of vegetable oil (Putt 1997).

Helianthus includes 50 species that are native to the Americas and found in the United States. Taxonomic complexity has resulted from natural hybridization, difference in ploidy levels, and environmental interactions. Seiler and Rieseberg (1997) provided an extensive review of germplasm resources in sunflower. The annual species, *H. annuus* (L.), includes plants variable for height, head diameter, number of days to flowering, photoperiod responses, and environmental adaptation. This wealth of diversity has enabled the development of cultivars for ornamental and cut flower uses. Worldwide distribution of sunflowers has been enhanced by the beauty and allure of the flowering heads. The cut flower industry is the most recent extension of sunflower utilization. The ongoing goal of sunflower research at Western Kentucky University is to identify the genotypes and environmental conditions that are suitable for sunflower production in South Central Kentucky and that produce floral characteristics sought by area gardeners and florists. The present study objective was to observe the variability in cut flower related traits and to compare the relative contributions of genetics and environments to that variation.

MATERIALS AND METHODS

Evaluation data were attained from three local experiments and from information provided by the seed companies. In 2004, 18 sunflower cultivars were grown at Bowling Green (BG1). In 2011, 18 cultivars, including 13 from the 2004 study (Table 1), were grown at Bowling Green (BG2) and in Owensboro (OB), Kentucky. The design was a randomized

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Table 1. Company sources for seeds of sunflower cultivars evaluated in different environments.

Cultivars	Environments: BG1	BG2 and OB[1]
	Company seed sources[2]	
Moulin Rouge	J	J
Ruby Eclipse	V	V
Panache	V	
Pacino Gold	J	
Giant Greystripe	J	VS
Velvet Queen	J	J
Teddy Bear	J	J
Apricot Twist	V	TS
Peach Passion	V	V
Valentine	J	V
Sunrich Lemon	J	J
Sunrich Orange	J	J
Double Dandy	V	
Sunbright	J	J
Moonbright	J	
Sunbeam	J	J
Soraya	J	
Ikarus	J	ST
Solar Eclipse		B
Little Becka		J
Italian White		PS
Full Sun		
Early Russian		V

[1] Environments: BG1, Bowling Green 2004; BG2 and OB, Bowling Green and Owensboro 2011.
[2] Seed companies: J-Johnny's, V-Vesey's, VS-Victory's Seeds, ST-Swallowtail, TS-Territorial Seed, B-Burpee, PS-Penya Seed.

complete block with three replications. Each entry consisted of one 10-plant row with 30 cm spacing within the row and 60 cm spacing between rows. A complete fertilizer (N 19%, P_2O_5 19%, K_2O 19%) was incorporated prior to seeding at the rate of 885 kg ha^{-1}. Weed control was obtained by a pre-emergence application of pendimethalin (1.12 kg A.I. ha^{-1}) to control grass weeds, plus limited hand weeding. Although descriptive data provided by the seed companies reflected observations from different locations, the data were included in the evaluations (Table 2). The cultivars were selected to represent dwarf, normal, and large forms of *H. annuus*.

Plant development data were collected from emergence through head harvest. Heading and flowering data were taken weekly during the flowering season. At each harvest, all flowering heads were removed, counted, and classified as marketable or non-marketable. Classification of marketability was based on head deformity, pest damage, over maturity, or other undesirable characteristics.

Data were subjected to analysis of variance procedures to determine whether environments differed significantly and to compare cultivar performance within environments. Linear correlation was used to compare cultivars for consistency in the expression of characteristics over environments (Steel and Torrie 1980). Consistency expression of a characteristic in different environments is evidence of a degree of genetic control. Cultivar popularity evaluations and vase life observations were based upon three freshly cut flowering heads placed in a vase containing commercial strength 'Flora-life' and water maintained at room temperature (approximately 22°C). Individuals from the University and its greater community rated their personal preferences on a subjective scale. Vase life was based on the number of days to petal drop.

RESULTS AND DISCUSSION

Combinations of specific cultivars and environments and company sources of seeds are presented in Table 1. Comparisons of

Table 2. Average and range of sunflower cultivars for selected criteria within different environments.

Criteria	Environments[1] BG1 Range[2]	BG2 Range	OB Range	SC Range
Emergence (%)	NA[3]	56 (13–87)	59 (23–93)	NA
Plants/cultivar	NA	17 (4–26)	18 (7–28)	NA
Days to flowering (DTF)	58 (42–75)	62 (54–77)	60 (54–76)	78 (55–120)
Height at flowering (m)	1.1 (0.6–1.8)	1.5 (0.7–2.7)	1.8 (0.5–3.0)	1.4 (1.0–2.0)
Heads/plant	14 (1–40)	23 (1–62)	36 (1–101)	NA
Marketable heads/plant	NA	10 (0–28)	27 (1–73)	NA
Non-marketable heads/plant	NA	13 (0–34)	9 (0–28)	NA
Head diameter (cm)	7 (4–13)	8 (4–13)	8 (5–14)	13 (9–25)
Stem diameter (cm)	NA	2.7 (1–5)	3.1 (1–6)	NA

[1] Environments: BG1, Bowling Green 2004; BG2 and OB, Bowling Green and Owensboro 2011, SC, Seed company information provided with cultivar seeds.
[2] Range among cultivars.
[3] NA, data not available.

Table 3. Linear correlation coefficients (r) and coefficients of determination ($r^2 \times 100$) between sunflower cultivar performances in different environments.

	Environments[1]											
	BG1 vs. BG2		BG1 vs. OB		BG1 vs. SC		BG2 vs. OB		BG2 vs. SC		OB vs. SC	
Criteria[2]	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2
Plants/cultivar	NA[2]		NA		NA		0.62**[3]	38	NA		NA	
Days to flowering (DTF)	0.74**	55	0.76**	58	0.07	0	0.80**	64	0.29^{NS}	8	0.07^{NS}	0
Height at flowering	0.75**	56	0.76**	58	0.71**	50	0.96**	92	-0.34^{NS}	12	-0.31^{NS}	10
Heads/plant	0.93**	86	0.74**	55	NA		0.89**	79	NA		NA	
Marketable heads/plant	NA		NA		NA		0.86**	74	NA		NA	
Non-marketable heads/plant	NA		NA		NA		0.96**	92	NA		NA	
Head diameter	0.85**	72	0.76**	58	0.45^{NS}	20	0.82**	67	0.41^{NS}	17	0.05^{NS}	0
Stem diameter	NA		NA		NA		0.63**	40	NA		NA	

[1] Environments: BG1, Bowling Green 2004; BG2 and OB, Bowling Green and Owensboro 2011; SC, Seed company information provided with cultivar seeds.
[2] NA, data not available.
[3] NS and ** indicate non-significant ($P > 0.05$) and highly significant ($P < 0.01$); respectively.

environments for expression of a given trait were based upon cultivars common to the environments being compared. Averages and ranges in cultivar performance for the various criteria are included in Table 2. Linear correlations (r) and coefficients of determination (r^2) given in Table 3 indicate the level of consistency of cultivars in expression of a trait over the environments.

Quantitative data on seedling emergence were obtained for BG2 and OB. Both environments had low emergence, but the averages (56 and 59%) and cultivar ranges (13–87 and 23–93%) were similar. In addition, the average number of plants in the two environments did not differ significantly ($P > 0.05$). The significant correlation (0.62) and magnitude of the coefficient determination (38%) indicated that both the environments and cultivars contributed to seedling emergence and plant survival. For the existing conditions, the environmental effects were greater than the genetic effects.

It was observed that heavy rainfall following planting resulted in soil crusting and, consequently, lowered seedling emergence. Gay, et al. (1991) reported that temperature is the most important environmental factor influencing germination of sunflowers and that the optimum is near 26°C. Germination is affected by seed characteristics including dormancy and aging, moisture content, oil composition, seed size, and seed shape. In the present study, attempts to correct stand differences were unsuccessful because replants were overshadowed and rarely attained full development. A more effective approach would be planting at higher density and thinning to the desired stand.

Days to flowering (DTF) did not differ significantly ($P > 0.05$) for BG1, BG2, and OB; however, these environments had significantly lower DTF than those provided by seed companies (SC). Correlations for DTF were positive and highly significant for all comparisons except those involving SC which were non-significant ($P > 0.05$). These high correlations for DTF across environments support the results reported by Russell (1953) who observed correlation coefficients in the range of 0.86 to 0.91 for DTF.

Average plant height at the time of flowering for cultivars in the three experiments and seed catalog data ranged from 1.1 to 1.8 m and was significantly lower for BG1 than for the other environments. Skoric (1988), as cited by Seiler (1997), reported that stem length of commercial sunflowers ranged from 0.5 to 5.0 m with most cultivars included between 1.6 to 1.8 m.

High correlations and determinations for cultivars over environments for both days to flowering and height at flowering were evidence of both genetic and environmental influences on flowering. Determination coefficients near 50% suggest that environmental and genetic effects were about equal, and that flowering in sunflowers is complex and variable. In a review of floral initiation, Seiler (1997) reviewed research results in which different genotypes responded as short-day, long-day, or day-neutral. Doyle (1975) reported that floral initiation was influenced more by temperature than by photoperiod.

Table 4. Floral characteristics of sunflower cultivars receiving high personal preference ratings as cut flowers.

Cultivars (alphabetical)	Flower color	Head[1] diameter (cm)	Vase life[1] (days)
Ikrarus	light yellow	5	11
Little Becka	red/orange	5	11
Moulin Rouge	deep burgundy	6	8
Sunbeam	golden yellow	12	13
Valentine	pale yellow	6	15
Velvet Queen	burgundy red	7	13

[1] Data are averages for BG2 and OB environments.

Total number and marketable heads/plant were significantly ($P < 0.05$) higher in OB than in BG1 and BG2, whereas non-marketable heads were more frequent in BG2 than in OB ($P < 0.05$). Non-marketable heads included cultivar-specific deformities, pest induced injuries, and over-maturity. Some cultivars did not produce symmetrical heads desired by florists. Although sunflowers are susceptible to numerous insects and no pest control measures were applied, likely because the low intensity of sunflower production and isolation of environments were not conducive to pest buildup. Overmaturity was the major factor in head deterioration, indicating that weekly harvest intervals were too long. Correlations of head production by cultivars over environments BG1 vs. BG2, BG1 vs. OB, BG2 vs. OB were highly significant ($P < 0.01$) for total heads. Marketable head production by cultivars was significantly correlated ($P < 0.01$) between BG2 and OB; the results were similar for non-marketable heads. Average head diameter for cultivars in BG1, BG2, and OB ranged from 7 to 8 cm and were not significantly ($P > 0.05$) different, but appeared to differ from the 13 cm average diameter reported for the SC environment. Using a compilation of seed company information was useful in the present study. However, new cultivars should be grown on small scale for observation on plant adaptation and head characteristics before making a large investment.

Average stem diameter did not differ significantly between BG2 and OB; however, cultivars varied within the environments and that variation was significantly correlated between the two environments. Cultivars with larger stems usually had greater heights, fewer but larger heads, and occasional lodging. Tall robust plants were more difficult to harvest for cut flowers.

Personal preferences for cultivars as cut flowers are presented in Table 4 and Figure 1. Cultivars receiving high ratings for BG1, BG2, and OB included more cultivars with the traditional yellow color; however, 'Moulin Rouge,' a highly preferred cultivar, had a deep burgundy color. With exception of 'Sunbeam,' the preferred cultivars had head diameters smaller than the average for all cultivars. Preferences appeared to be based upon overall appearance rather than specific color or head size.

Vase life of cut flowers is critical to economical production and marketing. The present cultivars exhibited vase life range of 1 to 15 days before post-harvest petal wilt occurred. Most of the cultivars had a vase life of 11 to 12 days when placed in a commercial strength solution of water and Flora-life at room temperature (approximately 22°C). In the harvest procedure only fully open heads were harvested leaving partially open heads for the next weekly harvest. This delay consumed a portion of the vase life or resulted in overly mature heads that were classified as non-marketable. Study is needed to determine the earliest stage at which heads can be harvested without impairing normal development. Extension of vase life would enable cut flowers to be shipped greater distance and be enjoyed for longer periods.

SUMMARY

The results indicate that *H. annuus* sunflowers were adapted to the soils and other environmental conditions of South Central Kentucky. Cultural practices applicable to commonly grown garden and field crops were suitable for sunflower production. Expressions of plant and floral characteristics were influenced by both cultivars and environments. Head production was sufficient to support an economical enterprise, but improvements in management practices are needed. The weekly harvest schedule resulted in many heads becoming too mature and non-marketable. More frequent harvests would increase marketable heads and extend shipping qualities and vase life. Cut flowers from the different cultivars were largely pleasing and acceptable by both lay and professional individuals. The most desirable characteristics of the plants included smaller heads and longer stems. These traits were exhibited by cultivars

Figure 1. Visual characteristics of sunflower cultivars receiving high personal preference ratings as cut flowers.

included in the study; however, their expression could be enhanced by higher planting densities and earlier head harvests. Collectively, the results indicate that sunflower cut flowers are potentially a profitable crop for Central Kentucky producers.

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Lady Beetle Composition and Abundance in Sweet Corn Bordered by Pasture, Buckwheat or Sunflower Companion Plantings

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ABSTRACT

To determine the effect habitat management/border plantings have on beneficial insect diversity and abundance in agricultural crops, sweet corn, *Zea mays* L. 'Garrison®', was grown in replicated plots on Kentucky State University's Agricultural Research and Demonstration Farm. Each 25 m × 12 m plot was bordered on each length by a 2 m wide border of un-mowed pasture, buckwheat (*Fagopyrum esculentum* Moench), or dwarf sunflower (*Heliathus annuus* L. var. 'Big Smile'). Yellow sticky traps 15 cm × 15 cm were used to capture lady beetles weekly through anthesis. Pink lady beetle, *Coleomegilla maculata* (DeGeer); Asian lady beetle, *Harmonia axyridis* (Pallas); spotless lady beetle, *Cycloneda munda* (Say); and seven spotted lady beetle, *Coccinella septempunctata* L. were caught in this study. The pink lady beetle was the most abundant species overall in all three borders and in the sweet corn plots with 79% and 94%, respectively. There were greater numbers of pink lady beetles in buckwheat ($P < 0.00001$) and sunflower ($P = 0.0064$) borders than pasture borders. However, there were no differences among populations of any of the lady beetle species in any of the sweet corn plots. Pink lady beetles were more numerous in sweet corn bordered by buckwheat ($P \leq 0.05$), sunflower ($P < 0.0001$) and pasture borders ($P \leq 0.03$) than in the borders themselves.

KEY WORDS: Lady beetles, conservation biological control

INTRODUCTION

Reliance on insecticides for control of insect pests in sweet corn has caused concern for the development of insecticide resistance (Sparks 1981) and mortality of beneficial insects and other non-target arthropods (Musser and Shelton 2003), including honeybees foraging in intensively treated crops (Johnson et al. 2006). Other potential problems involve worker exposure to insecticides during mixing, application and commodity harvest, surface water and groundwater contamination, and the sustainability of conventional production systems (National Research Council 1989, 1996). Thus, there is a need for more ecologically sound pest management systems and decreased emphasis on insecticide applications.

Many researchers have suggested that non-crop vegetation present in crop field margins can serve as refuges and attract populations of predaceous arthropods and parasitoids, as well as increase the diversity of all arthropods (Dennis and Fry 1992). In most cases, predaceous arthropods such as ground beetles, syrphid flies, and spiders have been studied in crop-border systems (van Emden 1965; Pollard 1968; Altieri and Whitcomb 1980; Sunderland and Samu 2000).

Farming practices utilizing ecologically based insect pest control offer ways to potentially lower input costs, decrease reliance on insecticides and nonrenewable resources, increase biodiversity, and obtain premium prices for agricultural commodities compared to those conventionally produced (Greene 2001). However, as farmers attempt to establish alternative agricultural enterprises, a major constraint to the adoption of sustainable practices is the lack of research-backed information on ecologically based insect management and control strategies, especially for ear pests of sweet corn.

Lady beetles have been recognized as important predators in agricultural systems. In general, lady beetles have been reported to be attracted to annual flowering plants including sunflower (*Heliathus annuus* L.) and the exotic buckwheat (*Fagopyrum esculentum* Moench) (Dufour 2000; Ambrosino et al. 2006; Adedipe and Park 2010; Woltz et al. 2012). However, additional information concerning lady beetles and other beneficial insects is needed in conjunction with border rows providing floral resources so that effective and sustainable pest control options can

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Table 1. Species of lady beetles caught in buckwheat, sunflower and pasture borders during 2009 at the Kentucky State University Research and Demonstration Farm in Franklin County Kentucky.

Pink lady beetle, *Coleomegilla maculata* (DeGeer)
Asian lady beetle, *Harmonia axyridis* (Pallas)
Spotless lady beetle, *Cycloneda munda* (Say)
Seven-spotted lady beetle, *Coccinella septempunctata* L.

be identified and employed. Development of conservation biological control tactics such as habitat management may be expected to boost small farm income and benefit small farm communities. Thus, the objective of this research was to determine the effect habitat management/border planting has on lady beetle diversity and abundance in buckwheat, sunflower and pasture border rows and the sweet corn planted between pairs of each of these border types.

MATERIALS AND METHODS

Sweet corn, *Zea mays* 'Garrison®', was planted on 19 June 2009 using conventional farming practices in 25 m × 12 m plots on Kentucky State University's Agricultural Research and Demonstration Farm in Franklin County, Kentucky. Each sweet corn plot was bordered on each side of its length by a 2 m wide border of one of three treatments. The three treatments were 1) un-mowed pasture, 2) buckwheat (*Fagopyrum esculentum* Moench) and 3) dwarf sunflower (*Heliathus annuus* L. var. 'Big Smile'). Buckwheat borders were planted on 7 July using a Tye® Drill Planter (Lockney, TX). Sunflower borders were planted on 7 and 8 July using an Earthway® Garden Push Planter (Bristol, IN). Pasture borders were mowed at the time of buckwheat and sunflower planting, but remained unmowed for the remainder of the study. Pasture borders were composed primarily of tall fescue, *Festuca arundinacea* (Scop.) Holub; johnsongrass, *Sorghum halepense* (L.); orchard grass, *Dactylis glomerata* L.; giant foxtail, *Setaria faberi* Herrm; and smooth pigweed, *Amaranthus hybridus* L. A randomized complete block design with five replicates was used and all plots were separated by 25 m.

Yellow sticky traps 15 cm × 15 cm were used to capture lady beetles in August during sweet corn anthesis. Two traps were deployed at canopy height midway between the edges and equidistant from the ends of each border plot. Four traps were deployed in each sweet corn plot at ear height, one in the center of each plot quadrant. Traps were changed weekly through anthesis. Sticky traps were placed individually in Ziploc® plastic bags, labeled, and transported to the laboratory for insect identification and enumeration.

Population data for each lady beetle species were analyzed using ANOVA and Fisher's Protected LSD procedures in CoStat Statistical Software (CoHort Software 2006). Numbers of pink lady beetles, *Coleomegilla maculata* (DeGeer), were further analyzed according to habitat type using a *t*-test using the same statistical software. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Pink lady beetle, *Coleomegilla maculata*; Asian lady beetle, *Harmonia axyridis* (Pallas); spotless lady beetle, *Cycloneda munda* (Say); and the seven spotted lady beetle, *Coccinella septempunctata* L. were the lady beetle species caught in this study (Table 1). The pink lady beetle was the most abundant species in all three border types and the sweet corn plots with 79% and 94%, respectively.

There were greater numbers of pink lady beetles in buckwheat than the pasture and sunflower borders on 14 August ($P < 0.00001$), and a greater number in both buckwheat and sunflower borders than pasture borders on 20 August ($P = 0.0064$) (Table 2). These results are similar to those reported by Woltz et al. (2012) for buckwheat and pasture strips bordering soybeans in southern Michigan. There were no differences in abundance of Asian lady beetle, spotless lady beetle or seven spotted lady beetles on any of the three sampling dates. Results of the present study contrast with those reported by Adedipe and Park (2010) where the Asian lady beetle was more attracted to sunflower in laboratory olfactometer studies than any of 8 other species of flowering plants. Unfortunately, they did not report the variety of sunflower used in their study that could conceivably have affected the results of their study.

Table 2. Average number of lady beetles caught in buckwheat, sunflower and pasture border rows.

Date	Treatment	Pink[a] lady beetle	Asian lady beetle	Spotless lady beetle	Seven spotted lady beetle
August 14	Buckwheat	8.5 a	0.35a	0.55a	0.05a
	Sunflower	1.75b	0.30a	1.15a	0.05a
	Pasture	2.85b	0.15a	0.85a	0.00a
	N	5	5	5	5
	F value	16.36	0.90	0.74	0.49
	P value	0.00001***	0.41	0.48	0.62
August 20	Buckwheat	6.55a	0.20a	0.35a	0.1a
	Sunflower	5.65a	0.35a	0.40a	0a
	Pasture	1.8b	0.20a	0.25a	0a
	N	5	5	5	5
	F value	5.57	0.64	0.26	2.07
	P value	0.0064**	0.52	0.76	0.14
August 27	Buckwheat	4.0a	0.0a	0.1a	0.0a
	Sunflower	3.05a	0.1a	0.3a	0.0a
	Pasture	3.75a	0.05a	0.2a	0.05a
	N	5	5	5	5
	F value	0.32	1.07	0.98	0.99
	P value	0.73	0.35	0.38	0.37

[a] Means followed by different letters are significantly different.

Pink lady beetles were the dominant species in sweet corn bordered by buckwheat, sunflower and pasture borders. However, there were no differences among populations of any of the lady beetle species in any of the sweet corn plots bordered by any of the three border types (Table 3).

Pink lady beetles were significantly more numerous in sweet corn bordered by buckwheat than in buckwheat borders on 20 August ($P \leq 0.05$) (Table 4). On 14 August pink lady beetles were more numerous in sweet corn bordered by sunflower than sunflower borders ($P < 0.00$) (Table 4). Sweet corn bordered by pasture borders had significantly greater numbers of pink lady beetles than pasture borders on 14 August ($P = 0.03$) and 20 August ($P < 0.00$) (Table 4). There was a trend toward higher numbers in sweet corn on all three sampling dates for all border types. Interestingly, Woltz et al. (2012) did not find higher numbers of any lady beetle species in soybean plots bordered by buckwheat in southern Michigan.

Table 3. Average number of lady beetles caught in sweet corn bordered by buckwheat, sunflower and pasture border rows.[a]

Date	Treatment	Pink lady beetle	Asian lady beetle	Spotless lady beetle	Seven spotted lady beetle
August 14	Corn-Buckwheat	8.7a	0.35a	0.65a	0.05a
	Corn-Sunflower	6.8a	0.25a	0.70a	0.00a
	Corn-Pasture	8.5a	0.15a	0.40a	0.00a
	N	5	5	5	5
	F value	1.01	0.77	0.35	0.99
	P value	0.7	0.47	0.71	0.37
August 20	Corn-Buckwheat	12.6a	0.0a	0.1a	0
	Corn-Sunflower	12.0a	0.0a	0.05a	0
	Corn-Pasture	13.5a	0.05a	0.2a	0
	N	5	5	5	5
	F value	0.17	0.99	1.32	-
	P value	0.84	0.37	0.27	-
August 27	Corn-Buckwheat	5.6a	0a	0.1a	0
	Corn-Sunflower	6.4a	0a	0.2a	0
	Corn-Pasture	6.5a	0.05a	0.05a	0
	N	5	5	5	5
	F value	0.26	0.99	1.68	-
	P value	0.78	0.37	0.19	-

[a] Means followed by the same letters are not significantly different.

Table 4. Average number of pink lady beetles caught in buckwheat, sunflower or pasture border rows compared with the numbers caught in sweet corn bordered by each border type.

	8/14		8/20		8/27	
	Corn/Buckwheat	Buckwheat	Corn/Buckwheat	Buckwheat	Corn/Buckwheat	Buckwheat
Mean	8.70	8.50	12.55	6.55	5.55	4.00
N	5	5	5	5	5	5
P(T <= t) two-tail	0.94		0.05*		0.46	
	Corn/Sunflower	Sunflower	Corn/Sunflower	Sunflower	Corn/Sunflower	Sunflower
Mean	6.75	1.75	12.00	5.65	6.40	3.05
N	5	5	5	5	5	5
P(T <= t) two-tail	0.00***		0.08		0.12	
	Corn/Pasture	Pasture	Corn/Pasture	Pasture	Corn/Pasture	Pasture
Mean	8.50	2.85	13.50	1.80	6.55	3.75
N	5	5	5	5.00	5	5
P(T <= t) two-tail	0.03**		0.00***		0.25	

* $P \leq 0.05$, ** $P \leq 0.03$, *** $P < 0.01$.

Kernel damage was not quantified; however there appeared to be no reduction in damage to sweet corn ears. Additional field experiments should be performed in which flowering of buckwheat and sunflower occurs before, and not the beginning of, the onset of sweet corn anthesis. Interplanting the buckwheat and sunflower in narrow strips within the sweet corn plots should also be investigated.

In conclusion, the pink lady beetle was the most abundant species in all three border types and the sweet corn plots. Buckwheat and sunflower borders contained more pink lady beetles than pasture borders and pink lady beetles in sweet corn were more abundant than in borders. Based on these observations it was concluded that buckwheat, sunflower and pasture border rows have little influence on lady beetle populations in sweet corn when planted in the manner described in this study. Field experiments using other species of flowering plants, for example native perennials, should be performed. In addition, planting strategies to influence longer flowering periods of border plants could enhance the abundance of lady beetles, other predatory insects, and parasitoids in sweet corn when ear pests are present.

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Genetic Diversity in Kentucky Spicebush Populations using Simple Sequence Repeat Markers

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ABSTRACT

Spicebush (*Lindera benzoin* L.) is a small native shrub that grows in the moist, understory areas of Appalachia and has potential as a new niche crop for small farmers. Native Americans and early settlers used this plant traditionally as a tea, and used the berries for jam, to spice food, and possibly for health benefits. Native spicebush patches may serve an important role in forest ecosystems in terms of fruit production for animals, soil erosion control, and enhancing insect biodiversity. Spicebush may also serve to hold ecological niches by outcompeting invasive plants compared to those in unchallenged areas. Genetic diversity of native spicebush populations in Kentucky has not been examined. The objective of this study is to determine the genetic diversity in spicebush populations in Kentucky using simple sequence repeat (SSR) DNA marker systems. Leaf samples were collected from 120 spicebush plants in the forests at the Kentucky State University Environmental Education Center (EEC), at a location near the Kentucky River, and at Cove Spring Park. DNA was extracted using the DNAmite Plant Kit. Primers A7, A115, B105, B122, C4, and C10 were used to amplify SSR products that were separated with a 3130 Applied Biosystems capillary electrophoresis system. The software program Power Marker was used to examine genetic relationships among genotypes. The SSR markers generated showed high genetic variation among the spicebush genotypes with the observed heterozygosity at 0.78. A number of selections with unique genotypes should be sampled and propagated for study in the KSU germplasm collection for potential cultivar development.

KEY WORDS: *Lindera benzoin* (L.), Microsatellites, Simple Sequence Repeat (SSR), Perennial shrub, Spicebush, Genetic Diversity

INTRODUCTION

Spicebush (*Lindera benzoin* L.) is a large, highly branched shrub or small tree. It is able to grow to over 12 feet tall and 15 feet wide. This plant is usually found as an understory plant in the forests of North America, especially in the eastern United States (Boyle 1980). Spicebush can be found near wetlands, woodlands, and in other shaded moist areas and in soils rich in limestone (Niesenbaum 1992). The leaves of the spicebush are one of the first to emerge in the spring or late winter compared to other plants. The leaves are obovate and alternate, and contribute to its name because of the aromatic spicy scent that comes from them as well as the berries and stems of the plant. Historically, the leaves of this shrub have been used for tea and medicine by Native Americans and early settlers (Anderson et al. 1992; Meuninck, 2007). The tea made from the leaves has been used to relieve pain, fever, arthritis, and breathing difficulties (Anderson et al. 1992; Meuninck 2007). The oil from the berries was sometimes used as a first aid treatment. Further research is needed to determine the effectiveness of these uses (Allison 2003). The berries have been used in jams, and are currently sold in combination with fruit such as blueberry and pawpaw to create flavors such as pawpaw spiceberry jam or blueberry spicebush jam. The berries have also been used as food flavoring by grinding the dried berries and using it as a seasoning on meat. The seeds, also known as drupes, are usually about 8 mm long, a bright cherry red color when mature, and contain a seed. The fruit matures in late summer or early fall (Tucker and Maciarello 1994; Meuninch 2007). Interestingly, spicebush has no significant disease or pest problems, which allows it to have the potential to be a new niche crop for small farmers.

Spicebush serves an important role in the ecosystems of forests. The fruit enhances biodiversity since it can be eaten by animals and insects, the root system in soils helps prevent soil erosion around streams and rivers, and biodiversity is enhanced from species that thrive specifically on spicebush such as the Spicebush Swallowtail (*Papilio troilus*) (Matlack 1994). With the unique food

and medicinal qualities, as well as the importance of this plant in the Kentucky ecosystem, spicebush is attractive to small farmers as a potential alternative crop, and as a plant for improving biodiversity in woodlands.

Microsatellites, or simple sequence repeats (SSRs), are a marker of choice for genetic diversity estimates, genetic mapping, and DNA fingerprinting (Wünsch and Hormaza 2002). SSRs are short (1–6 bp) tandem repeat DNA sequences flanked by unique, conserved DNA sequences. The relative random distribution of microsatellites in the genome, codominant inheritance, high level of reproducibility and transportability across laboratories make these markers useful for assessing genetic diversity as well as fingerprinting (Kijas et al. 1995; Wünsch and Hormaza 2002). SSR marker systems have been developed for a number of fruit species including pawpaw [*Asimina triloba* (L.) (Pomper et al. 2010)], hazelnut [*Corylus avellana* L. (Bassil et al. 2005)], blueberry [*Vaccinium corymbosum* L. (Boches et al. 2006)], peach [*Prunus persica* (L.) Batsch; (Aranzana et al. 2002)], cherry [*Prunus cerasus* L. (Cantini et al. 2001)], pear [*Pyrus communis* L. (Yamamoto et al. 2001)], and apple [*Malus sylvestris* (L.) Mill. (Hokanson et al. 1998, 2001)].

Spicebush can be found in a range of different light environments (Veres and Pickett 1982). There have been several previous limited studies assessing genetic diversity of spicebush in eastern Pennsylvania (Edwards and Niesenbaum 2007; and Mooney et al. 2010). Edwards and Niesenbaum (2007) developed 12 polymorphic SSR primer sets for examining genetic diversity in spicebush and Mooney et al. (2010) used these markers to examine genetic differences between spicebush in sun and shade environments. Genetic diversity of spicebush populations in Kentucky has not been examined. The long term goals of this research are to understand the cycling and spread of native spicebush patches as a part of Kentucky State University's mission and germplasm collection efforts in order to select genetically different varieties that can be grown by small farmers. Our objective was to determine the genetic diversity in spicebush populations in Kentucky using polymerase chain reaction (PCR) and SSR-DNA marker systems.

Table 1. GPS coordinates for sample locations.

Location	Coordinates
Cove Spring 1	38°13′08.39″N
	84°53′31.80″W
Cove Spring 2	38°13′11.24″N
	84°50′43.44″W
EEC 1	38°21′38.45″N
	85°00′44.07″W
EEC 2	38°21′35.32″N
	85°00′50.84″W
Kentucky River 1	38°08′19.10″N
	84°51′58.91″W
Kentucky River 2	38°08′18.11″N
	84°52′01.21″W

MATERIALS AND METHODS

Plant Material

Leaf samples were collected from spicebush plants in six spicebush populations in Kentucky using. Leaf samples were collected from spicebush plants in the forests at the Kentucky State University Environmental Education Center (EEC) in Henry County, Kentucky, Cove Spring Park in Frankfort, Kentucky, and a location near the Kentucky River near Frankfort, Kentucky (Table 1). Each population contained twenty individuals and two populations were sampled at each site.

DNA Extraction

DNA was extracted from the pawpaw leaves using the DNAMITE Plant Kit (The Gel Company, San Francisco, CA). About 1 ~ 2 cm^2 of young leaf tissue was used. The DNA concentration and a 260/280 nm absorbance ratio were determined with GeneQuantTM pro RNA/DNA calculator (GE Healthcare, Piscataway, NJ). All samples were stored at −80°C until needed.

SSR-PCR Amplification

The SSR-PCR amplification was performed with GoTaq Flexi DNA polymerase (Promega Co., Madison, WI). The reactions were set up follows: 4 µl of 5× colorless GoTaq Flexi buffer, 0.4 µl of 10 mM dNTPs solution, 2 µl of 25 mM MgCl2, 0.3 µl of 3 µM forward primer (fluorescence labeled with FAM) solution and 0.3 µl of 3 µM reverse primer (unlabeled) solution, 0.2 µl of 5 units/µl GoTaq DNA polymerase, 2 µl of diluted 1 ng/µl pawpaw DNA, and 10.8 µl of ddH2O

Table 2. The six loci tested, their forward and reverse sequences, and the repeat motif used in the SSR-PCR.

Locus	Primer sequence (5′–3′)	Repeat motif
A7	F:AAAACGGATCAGATACTCCC R:GCAGCATTATTGGGTTAGTG	$(AC)_{13}$
A115	F:AGGAGCTACCTCTGATTCTTGG R:TCACCCACATCTCAATATCATG	$(CT)_{19} + (AC)_{13}$
B105	F:ACAGGTCTTGACTTTGGGATAT R:GGATGGCTTATGGAGTGG	$(GA)_{11}$
B122	F:TGCTCAAGGAGAGATTCAAC R:CTCAGCCGAGTCTACTATCG	$(AG)_{17}$
C4	TTGGGTGGAGTTGATGAG R:ACGCATTATTGACAGCCTT	$(GAA)_{13}$
C10	F:TTCCTAAACCCTGTTGTAAAAC R:GCCAATCATGTGACTATTGTC	$(AAG)_{15}$

to bring the total volume to 20 µl. Six primer pairs were selected and labeled with FAM or HEX for use in this study:sb-A7, sb-A115, sb-B105, sb-B122, sb-C4, and sb-C10 (Table 2). The PCR amplifications were performed using the Endurance Series TC-512 Thermal Cycler (Techne Inc., Burlington, NJ). The PCR program consisted of an initial period of 94°C for 3 min, followed with 30 cycles of 40 sec denaturation at 94°C, 40 sec annealing at 56°C, and 30 sec extension at 72°C, and a final extension period of 10 min at 68°C. The PCR results were then stored at 4°C until analysis. Products were separated with ABI 3130 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) with GeneScanTM 500 LIZTM as internal size standard. Individuals were genotyped with GeneMapper version 4.0 software (Applied Biosystems Inc., Foster City, CA). At least two replicate amplifications were subjected to electrophoresis and analysis for each primer set.

Data Analysis

PowerMarker (Version 3.25) (Liu and Muse 2005) was used to calculate the observed number of alleles (nA), the observed heterozygosity (H_o), the expected heterozygosity (H_e) and polymorphic Information Index (PIC). The observed heterozygosity was calculated as the number of heterozygous genotypes at a given locus divided by the number of genotypes present at the locus. Gene diversity was defined as the probability that two randomly chosen alleles from the population are different. PIC was an estimate of the probability that the parental origin of an allele can be determined from the marker locus genotype in any given offspring. The equation for Ho, He, and PIC are given in the PowerMarker software manual. Genetic distance (D) between genotypes was computed as (1 − proportion of shared alleles (Bowcock et al. 1994). Cluster analysis of distance data were used to generate a dendrogram based on the matrix of the distances using unweighted pair-group mean analysis (UPGMA). The level of genetic similarity among cultivars and advanced selections was also determined by Nei's genetic distance (Nei 1978).

RESULTS AND DISCUSSION

All six of the SSR primer sets tested amplified SSR-PCR products in all popula-

Table 3. The six loci tested, the number of genotypes, the number of alleles, the allele size, the expected heterozygocity, the observed heterozygocity, and the PIC for all loci tested.

Marker	Genotype No.	Allele No.	Allele size (bp)	H_e	H_o	PIC
A115	75	30	160–248	0.92	0.85	0.91
A7	39	13	233–250	0.86	0.73	0.85
B105	87	27	238–292	0.93	0.88	0.93
B122	69	23	191–267	0.89	0.78	0.89
C4	38	13	222–247	0.87	0.60	0.86
C10	42	17	133–230	0.87	0.82	0.85
Mean	58	21	133–292	0.89	0.78	0.88

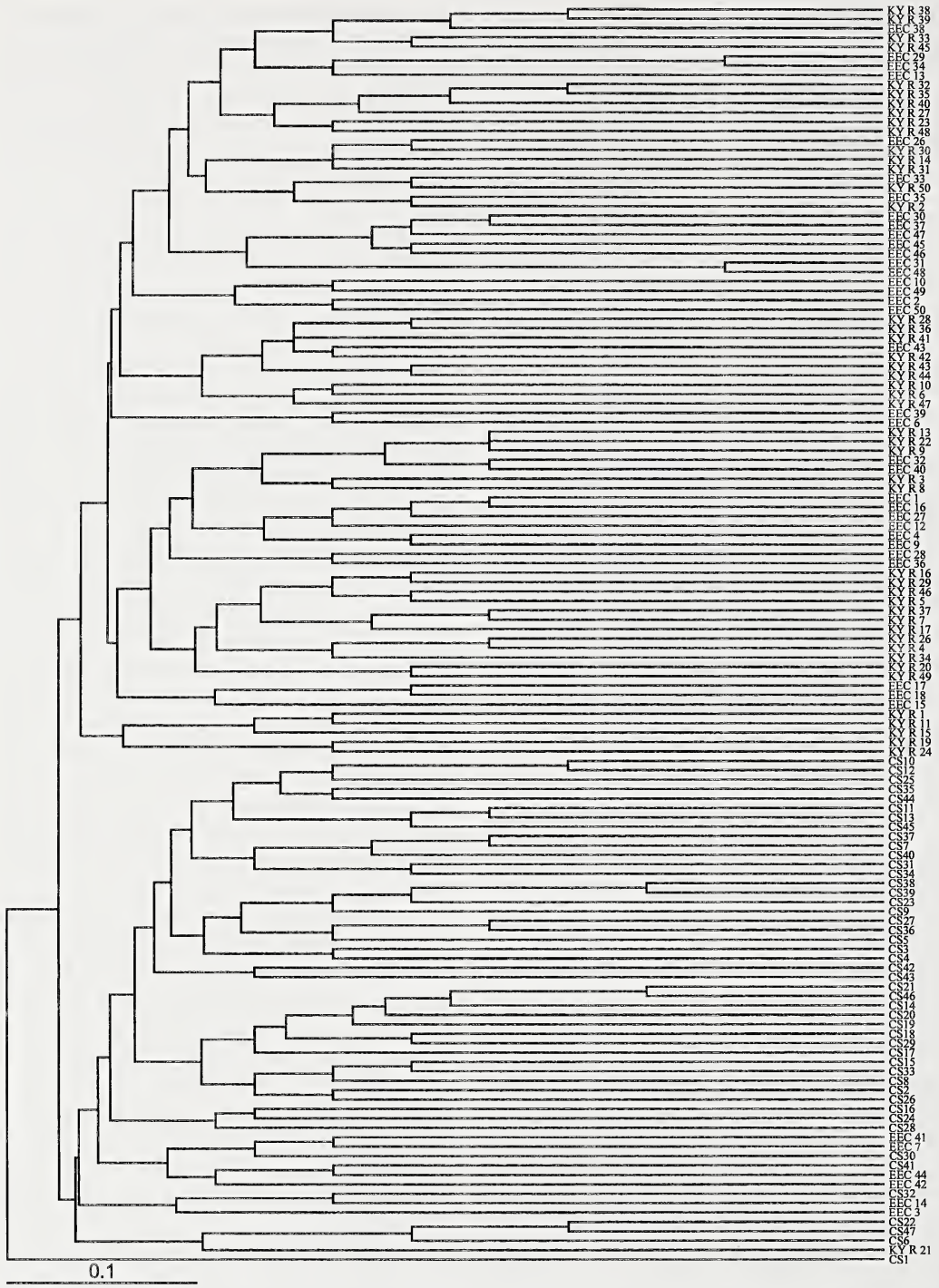

Figure 1. A dendrogram comparing genetic relationships of all the samples at all six patches at the three locations.

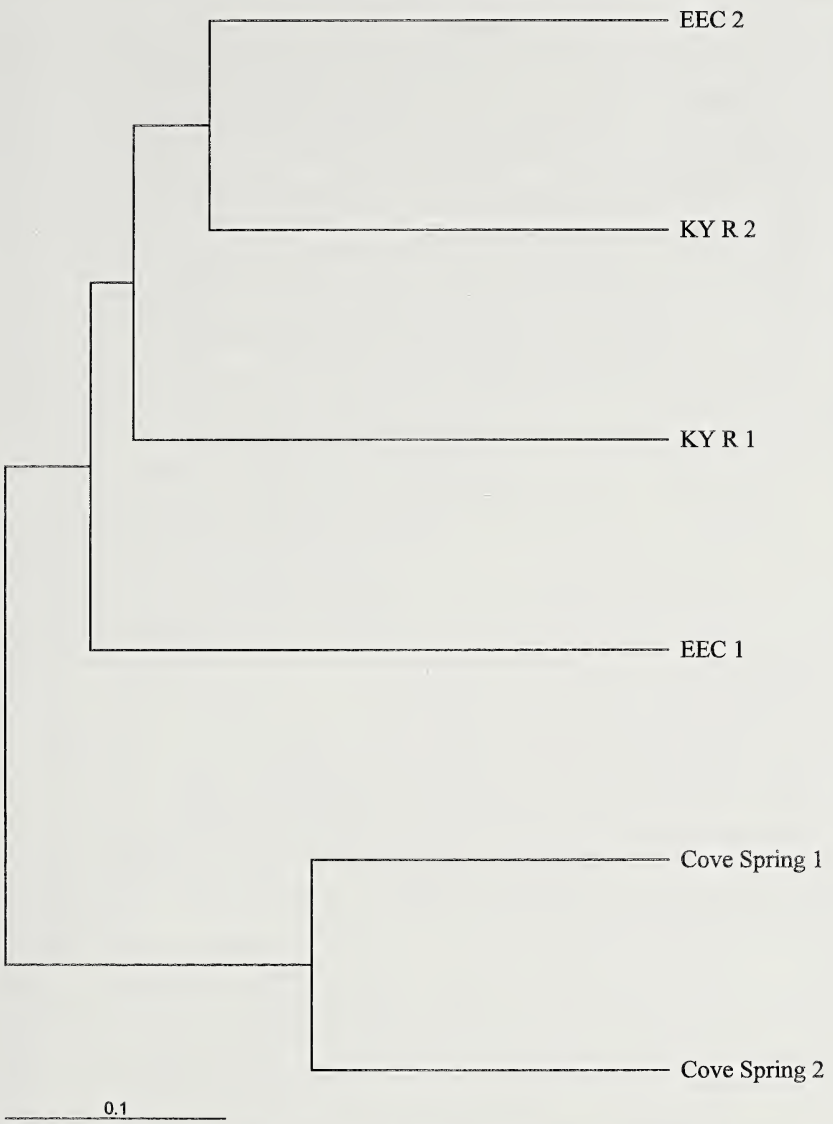

Figure 2. A dendrogram comparing the genetic relationships between the six patches.

tions examined. On average, each locus produced over 18 different alleles and 42 different genotypes. Allele size ranged from 133 to 292 base pairs and heterozygosity was high (H_e = 0.89; Table 3). When comparing populations, there was significant genetic diversity. The high average number of alleles per locus (21.0) is comparable to that in other woody perennial fruit and nut crop species such as 13.3 alleles per locus reported for hazelnut (Bassil et al. 2005) 12.1 for apple (Hokanson et al. 1998, 2001) and 9.1 for pear (Sisko 2009.). The H_e (0.89) in our study indicated high levels of genetic diversity among the spicebush populations examined. Edwards and Niesenbaum (2007) examined genetic diversity of 29 eastern Pennsylvania spicebush plants using 11 SSR primer pairs and found H_e ranging from 0.10 to 0.82, and alleles ranged from two to 16 per locus. Mooney et al. (2010) examined genetic diversity of 127 eastern Pennsylvania spicebush plants using 12 SSR primer pairs and found H_e ranging from 0.61 to 0.65, and alleles ranged from 3.7 to 4.3 per locus. The higher H_e determined for spicebush popula-

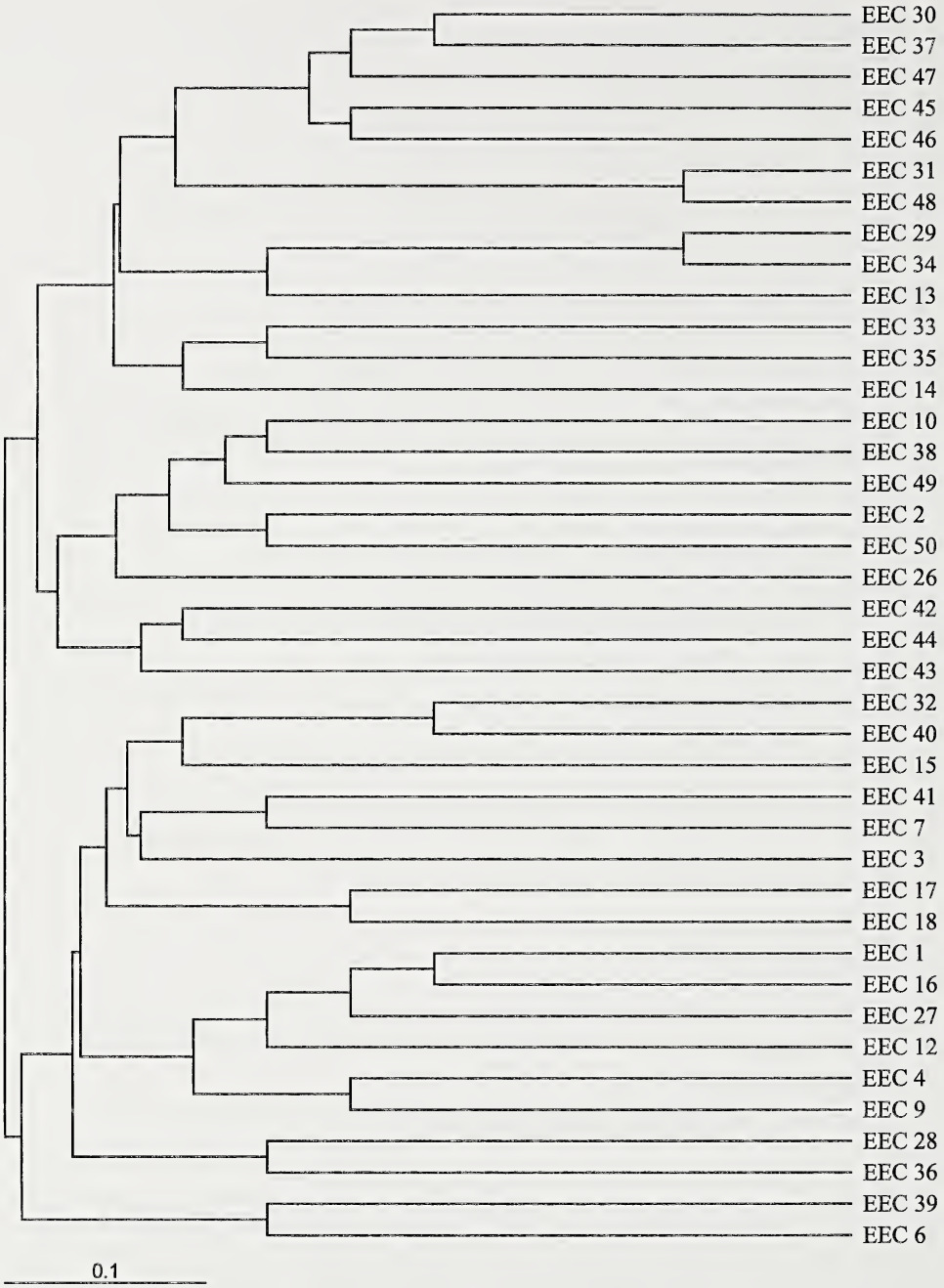

Figure 3. A dendrogram comparing the EEC location.

tions in our study likely reflects the wider sampling of populations separated by greater distance and therefore a more precise estimate of genetic diversity of spicebush in the wild.

UPGMA cluster analysis was used to generate a dendrogram (Figures 1, 2, 3, and 4). UPGMA analysis indicated the EEC and KYR populations were easily separated. Figure 1 shows there was a significant amount of diversity among the three locations. Figure 2 shows the genetic relationships between the six patches. The two Cove Spring patches

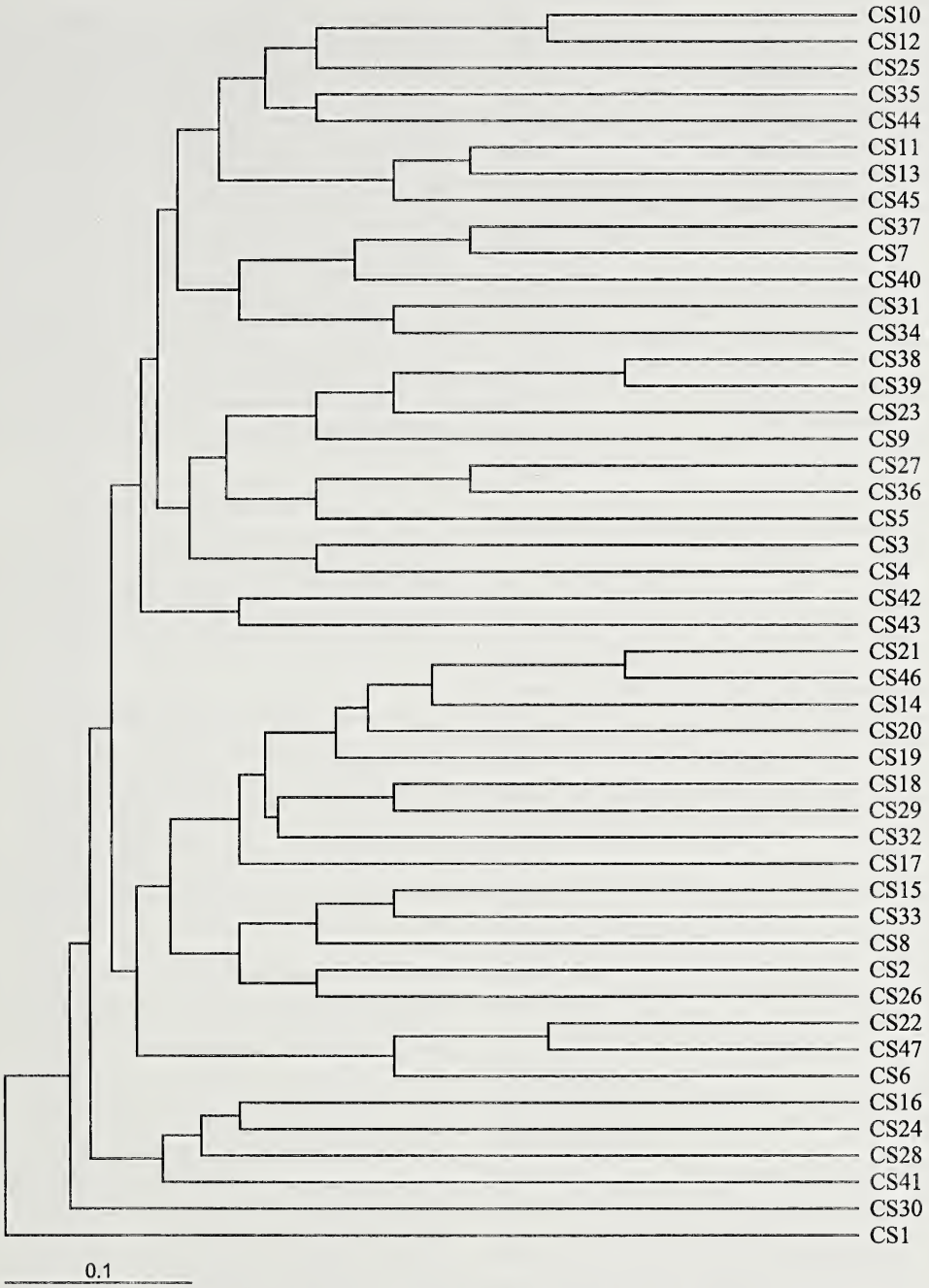

Figure 4. A dendrogram comparing the Cove Spring location.

were genetically similar. Conversely, the EEC2 population was closer genetically to the KYR 2 population than to EEC1; even though the two EEC populations were closer in proximity. Figure 3 shows the genetic relationships in the EEC populations. This population showed genetic diversity, but samples EEC31 and EEC48 and EEC29 and EEC34 were more similar. Figure 4 shows the genetic relationships in the Cove Spring populations. This population showed more similarities between samples than the

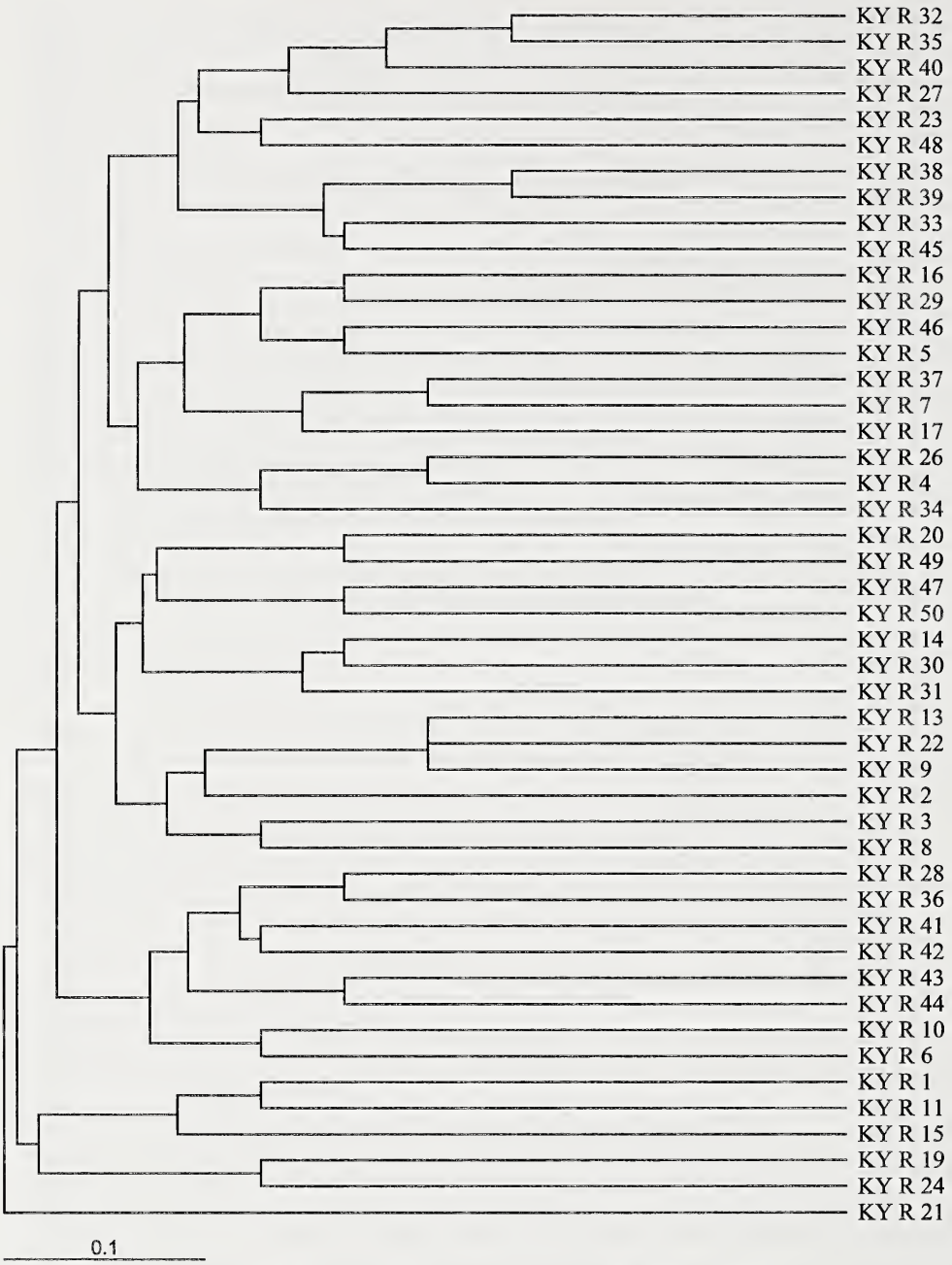

Figure 5. A dendrogram comparing the Kentucky River location.

EEC population. With the Cove Spring samples, CS22 and CS47, CS46 and CS21, CS38 and CS39, and CS10 and CS12 were similar. Figure 5 shows the genetic relationships in the Kentucky River populations. Interestingly, with this population, three samples were similar, KYR13, KYR22, and KYR9. Each of the three populations showed genetic similarities among samples.

In conclusion, there was significant genetic diversity observed in the spicebush populations examined. This is the first report of spicebush genetic diversity and relationships in Kentucky. The results of this study indicate

the six primers used should be useful in examining additional spicebush populations. For the establishment of spicebush varieties for small farmers in Kentucky, varieties with broad genetic diversity will be important for future insect and disease resistance when planted by farmers in larger monocultures that increase disease and pest pressure. Large fruit size will also be important to growers for spicebush to become a successful crop for small farmers. Future studies will include looking at fruit size among different populations. A number of selections with unique genotypes will be sampled and propagated for study in the KSU germplasm collection for potential cultivar development.

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The Pawpaw Peduncle Borer, *Talponia plummeriana* Busck (Lepidoptera: Tortricidae): A Pest of Pawpaw Fruit

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ABSTRACT

The pawpaw peduncle borer, *Talponia plummeriana* Busck (Lepidoptera: Torticidae), is a pest of pawpaw flowers often boring into the peduncle and causing flower drop. Here we document the first occurrence of this insect infesting ripe pawpaw fruit. Infested fruit that had been collected at the Kentucky State University Research and Demonstration Farm in Franklin County, Kentucky were dissected and small tan colored larvae with brown head capsules were discovered in the fruit. Pawpaw peduncle borer adults were reared from fruit held at room temperature in the laboratory.

KEY WORDS: *Asimina triloba* fruit pest, Pawpaw peduncle borer

INTRODUCTION

The North American pawpaw, *Asimina triloba* (L.) Dunal is a tree-fruit in the early stages of commercial production. It has had few reported pest problems. Pawpaws are native to mesic hardwood forests of 26 states in the eastern United States, including Kentucky (Lagrange and Tramer 1985; Chester et al. 1995) and surrounding states (Rheinhardt and Rheinhardt 2000; Larimore et al. 2003). Its full range extends from northern Florida to southern Ontario (Canada) and as far west as eastern Nebraska (Kral 1960). This small, deciduous tree may attain a height of five to ten m and tends to be found in patches (Layne 1996). Pawpaws are often found growing as understory trees in the deep, rich fertile soils of river-bottom lands (Kral 1960; Young and Yavitt 1987; Callaway 1990; Callaway 1993; Pomper et al. 2009). Pawpaw trees flower from April through May in Kentucky and are pollinated by flies and beetles (Faegri and van der Piji 1971).

One recognized pest, the pawpaw peduncle borer, *Talponia plummeriana* Busck, is a moth in the family Tortricidae. Pawpaw peduncle borer adults are approximately six mm in length with gray speckled wings and a wide copper band at the distal portion of the wings. The larvae have been previously documented to feed only on pawpaw flower peduncles (Bratch 2009). The larvae bore into the flower and peduncle eventually killing the flower.

In the orchards of the Kentucky State University Pawpaw Research Program, a small number of unripe and ripe pawpaw fruit showed evidence of feeding activity from an unknown fruit borer (Figure 1) causing extensive internal injury to the fruit (Pomper et al. 2008). Thus, the objective of this project was to determine the insect species responsible for damage to the fruit of pawpaw.

MATERIALS AND METHODS

Ripe pawpaw fruit were collected in mid-August from those that had fallen from trees in orchards located at the Kentucky State University Research and Demonstration Farm located in Franklin County, KY. Three fruit were placed into each of two one-liter plastic vegetable crispers (six total fruit) and incubated at room temperature in the laboratory until moths had emerged (Figure 2). We used the BugGuide Internet Identification Website (2010) and North American Moth Photographers Group: *Talponia plummeriana* (2007) to identify adults. Digital photographs of adults were sent to Dr. Charles Covell, Florida Museum of Natural History, McGuire Center for Lepidoptera and Biodiversity, Gainesville, FL for identification confirmation.

RESULTS AND DISCUSSION

Approximately 10 individual pawpaw peduncle borer adults were reared from the fruit. Adults emerged from fruit were identified as

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Figure 1. Damaged pawpaw fruit and young pawpaw peduncle borer larva.

the pawpaw peduncle borer (*T. plummeriana*) (Figures 3, 4).

Pawpaw peduncle borer moths have been previously collected in Henderson, Menifee, Powell, Wolfe, and Hart Counties, Kentucky (Kentucky Butterfly Net Database 2010; United States National Museum 2010). In most years, the damage is light (about one to five percent of fruit displaying damage). However, in some years there can be significant loss of flowers that can reduce fruit set (Jones et al. 1998).

While pawpaw peduncle borer damage is usually light, it does have the potential to reduce fruit yields significantly by flower and fruit damage. The incidence and degree of damage in papaw fruit orchards to flowers and fruits by this species needs to be further investigated. Pawpaw pests may become of greater interest as the number of pawpaw plantings or monocultures increase and control measures may need to be implemented.

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Figure 2. Damaged fruit with puparia present.

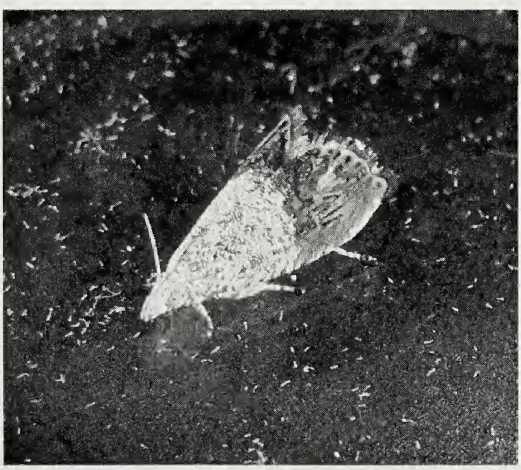

Figure 3. Pawpaw peduncle borer adult.

Figure 4. Pawpaw peduncle borer adults with U.S. quarter for scale.

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Pawpaw Patch Genetic Diversity, and Clonality, and its Impact on the Establishment of Invasive Species in the Forest Understory

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ABSTRACT

The pawpaw (*Asimina triloba*) is a native understory tree of 26 states of the Eastern and Midwestern United States. Pawpaw's genetic diversity and clonality in relation to this tree's ability to compete with local invasive species in Kentucky has not been examined. The objectives of this study were two-fold: to determine the genetic diversity and clonality displayed in seven native pawpaw patches located at the Kentucky State University Environmental Education Center (KSU-EEC), The Kentucky River, Cove Spring Park, and the KSU Research and Demonstration Farm in Franklin County using DNA microsatellite markers; and to determine if patches reduced the incidence of invasive species. Twenty-five trees from seven patches in the four different locations were sampled for genetic analysis. Leaf samples were extracted using the DNAmite Plant Extraction Kit and products from four microsatellite loci were analyzed using a 3130 Applied Biosystems Gene sequencer. String grids were created and invasive plants counted in three 5 meter squares in each of the patches and control plots outside of each patch. The number of plants for each invasive species within pawpaw patches was counted and compared to the control plots. Pawpaw patches displayed high genetic diversity among populations with some clonal sections in patches using DNA microsatellite makers, but no totally clonal patches. Amur honeysuckle (*Lonicera maackii*), garlic mustard (*Alliaria petiolata*), and winter creeper (*Euonymus fortunei*) were found in most locations; however, there was no significant difference in the incidence of invasive plants between the patches and the control plots. Pawpaw stem density and shading, among other factors may be important in the incidence of invasive plants within patches.

KEY WORDS: *Asimina triloba* (L) Dunal, Microsatellites, Simple Sequence Repeat (SSR), Pawpaw, Genetic Diversity, Forest Understory, *Lonicera maackii*, Amur Honeysuckle, Invasive Species, Kentucky State University, KSU

INTRODUCTION

Pawpaw [*Asimina triloba* (L) Dunal.] is an understory tree that is native to the Eastern and Midwestern United States. *A. triloba* can be observed in 26 states and ranges from northern Florida to southern Ontario, Canada, and as far west as Nebraska (Darrow 1975). Native pawpaw patches serve an important role in forest ecosystems and stream and rivers in terms of fruit production for animals, soil erosion control, enhancing insect biodiversity by bringing pawpaw exclusive species, and possibly resisting establishment of invasive plant species. Pawpaw is also in the early stages of commercial production. Unique native pawpaw germplasm could serve as future cultivars for growers or be used in KSU breeding efforts. *Asimina triloba* is a shade-loving understory tree which often forms dense patches in the understory of mixed mesophytic hardwood forests (Callaway 1990, 1993; Kral 1960; Young and Yavitt 1987). In the wild fruit set tends to be low possibly resulting from a lack of pollinators (beetles and flies) or due to limited resources, such as low light levels (Wilson and Schemske 1980; Faegri and van der Piji 1971). When flowers are successfully produced and pollinated, low light levels may impede the photosynthetic partitioning of energy to the fruit, reducing fruit production.

Asimina triloba will regularly generate many rootsuckers, potentially producing large clonal patches from an original seed (Pomper et al. 2010; Gould 1939). The propensity of individual pawpaw genotypes to form rootsuckers, and therefore more clonal patches, may be a genetic trait. Rootsuckers usually emerge near the original tree; therefore, increased stem density near some original trees may shade other understory plants. Because *A. triloba* is considered a self-incompatible tree, it further contributes to the low fruit production displayed in the wild. In the event fruit is produced, the large size of the seed makes it easy for its dispersal via mammalian activity (Cypher and Cypher

1999). However, *A. triloba* seed often fall victim to desiccation (Geneve et al. 2003) and freezing temperatures (Pomper et al. 2000). Genetically diverse native tree seedlings are an important component of forests because current seedling stocks will contribute to future survival, structure, composition, and economic value of the forest (Hutchinson and Vankat 1997).

An invasive shrub, Amur honeysuckle (*Lonicera maackii*), has become common throughout *A. triloba*'s native range. When *L. maackii* becomes established in the forest understory, it has a negative impact on native tree seedlings and herbs (Gould 1996; Collier 1997; Hutchinson and Vankat 1997; Trisel 1997). *Lonicera maackii* was introduced to North America in 1897 (Luken and Thieret 1996) and has been widely used as an ornamental (Hutchinson and Vankat 1998). This plant has escaped cultivation and grows wild in at least 24 states east of the Mississippi River (Trisel 1997). Due to a lack of native coevolved biological controls, *L. maackii* often grows unhindered (Schierenbeck et al. 1994). In forest edges (Luken and Goessling 1995) and areas of noticeable canopy disturbance, *L. maackii* has become a wide-spread problem by occupying forest niches that were home to a range of native plants (Yost et al. 1991; Demars and Runkle 1992). A number of additional invasive species [garlic mustard (*Alliaria petiolata*), winter creeper (*Euonymus fortunei*), and colts foot (*Tussilago farfara*)] are also commonly found in the Central Kentucky region and these species can also have a negative effect on native forest ecosystems.

Lonicera maackii has demonstrated the capabilities to reduced survival, growth, and reproduction of native herbs and trees. Also it has a negative bearing on species diversity, richness, and abundance of native plant species (Hutchinson and Vankat 1997; Collier et al. 2002; Hartman and McCarthy 2004). The ability of *L. maackii* to invade native plant ecosystems may be linked to its physiological traits. Invasive plants typically have a high allocation of energy to reproduction, plastic responses to the environment (branch structure), and primary productivity (Luken et al. 1995). Lonicera fruit ripening occurs in autumn when many native food sources of food are not available, thus large amounts of fruit/seed produced is scattered by birds (Ingold and Craycraft 1983). When high light levels are attained, primary productivity is high (Luken 1988; Luken et al. 1995). *Lonicera maackii* leaves are present for longer periods than native perennials (D. E. Trisel, unpublished data) allowing *L. maackii* to increase its net carbon gain (Harrington et al. 1989; Schierenbeck and Marshall 1993). Also some studies suggest that *L. maackii* may contain allelopathic chemicals which may inhibit native plant species from growing in its vicinity (Skulman et al. 2004; Dorning and Cipollini 2006; Trisel 1997). If *L. maackii* does produce allelopathic chemicals they likely enter the soil from leaching from decaying leaf material or secretion from its roots (Inderjit and Duke 2003).

Since both *A. triloba* and *L. maackii* hold the same niche in the forest understory (McEwan et al. 2009), it has been suggested that established *A. triloba*, along with other native understory species, could be used to prevent *L. maackii*'s movement into the forest understory (Hall 2003). It is important for *A. triloba* to maintain a high level of genetic diversity for its genetic advancement and reduction to disease vulnerability. The ability of some pawpaw genotypes to form many rootsuckers and thereby large clonal patches, could allow some pawpaw patches to hold an ecological niche in the forest understory when challenged by *L. maackii*.

Asimina species have large distribution ranges, which may enable them to retain significant genetic variation (Hamrick and Godt 1989; Lu et al. 2011). *Asimina triloba* is a diploid organism (Bowden 1948; Kral 1960) with protogynous and self-incompatible flowers (Wilson and Schemske 1980; Lu et al. 2011). However, because *A. triloba* is a clonal species genetic variation within populations may be low. Studies assessing genetic variation within *A. triloba* patches using inter simple sequence repeat (ISSR) markers have found that at least half of the patches were not clonal, containing at least 2 genotypes (Pomper et al. 2009b). Clonal pawpaw patches with genotypes that favor rootsucker formation could be more effective in holding forest niches against invasive species. Marker systems that have proven useful in the genetic

Table 1. GPS coordinates of all patches sampled.

Location	Coordinates	
Cove Spring A	38°13′7.82″N	84°50′31.11″W
Cove Spring B	38°13′7.06″N	84°50′31.44″W
EEC C	38°21′38.21″N	85°0′44.01″W
EEC D	38°21′35.96″N	85°0′50.58″W
KSU Farm E	38°7′12.70″N	84°53′2.81″W
KSU Farm F	38°7′11.71″N	84°53′1.99″W
Kentucky River G	38°8′19.10″N	84°51′59.30″W

assessment of *A. triloba* include: minisatellite probes (Rogstad et al. 1991), allozymes (Huang et al. 1997, 1998), random amplified polymorphic DNA (Huang et al. 2000, 2003), amplified fragment length polymorphism (Wang et al. 2005), intersimple sequence repeat (Pomper et al. 2003), and simple sequence repeat (Pomper et al. 2010).

Simple sequence repeats (SSRs), have become the marker of choice for genetic diversity assessment, genetic mapping, and DNA fingerprinting (Wünsch and Hormaza 2002). SSRs are short (1–6 base pairs) tandem repeat DNA sequences flanked by unique, conserved DNA sequences. The relative random distribution of microsatellites in the genome, codominant inheritance, high level of reproducibility, and transportability across laboratories make these markers useful for assessing genetic diversity, as well as fingerprinting (Kijas et al. 1995; Wünsch and Hormaza 2002). Microsatellite markers have not been used to determining clonality or assess genetic diversity pawpaw patches.

The objectives of this study were two-fold, to determine: 1) the genetic diversity and clonality displayed in seven native pawpaw patches located at the Kentucky State University Environmental Education Center, The Kentucky River, Cove Spring Park, and the KSU research and Demonstration Farm using DNA microsatellite markers; and 2) if patches, especially those that are clonal, reduced the incidence of invasive species.

MATERIALS AND METHODS

Plant Material

Leaf samples were collected from a total of seven different *A. triloba* patches at five different locations surrounding Frankfort, Kentucky (Table 1. GPS coordinates and map). From each patch, 25 leaf samples were collected from different stems located along a line transect ran through the middle of the longest portion of the patch. Samples collected from each population were placed into individual Ziploc bags with a tree identification number and placed into short term storage at 4°C until they were needed for DNA extraction.

DNA Extraction

DNA was extracted from the leaves using the DNAMITE Plant Kit (Gel CO., San Francisco). Approximately 1–2 cm^2 of leaf material was used. DNA concentration and the 260/280 nm absorbance ratio were determined using a GeneQuant™ *pro* RNA/DNA calculator (GE Healthcare Biosciences, Piscataway, NJ). All samples were then stored at −80°C for further use (Lu et al. 2011; Pomper et al. 2010).

SSR-Polymerase Chain Reaction (PCR) Amplification

PCR amplification for the SSR markers was performed using GoTaq Flexi DNA polymerase (Promega, Madison, WI). The reaction ingredients were as follows: 4 mL of 5× colorless GoTaq Flexi buffer, 0.4 mL of 10 mM dNTPs solution, 2 mL of 25 mM $MgCl_2$, 0.3 mL of 30 mM forward primer (fluorescence labeled with FAM) solution, and 0.3 mL of 30 mM reverse primer (unlabeled) solution, 0.2 mL of 5 units/mL GoTaq DNA polymerase, 2 mL of diluted 1 ng mL^{-1} pawpaw DNA, and 10.8 mL of ddH_2O to bring the total volume to 20 mL (Pomper et al. 2010). Six primers were chosen labeled with FAM/HEX for this study (Table 2). The PCR amplification was performed using an Applied Biosystems GeneAmp PCR System 9700 Techne thermal cycler (Carlsbad, CA). The program used for amplification was as follows: initial denaturation at a temperature of 94°C for 3 min, followed with 30 cycles of 40 sec denaturation at 94°C, 40 sec annealing at 56°C, a 30 sec extension at 72°C, and a final extension period of 10 min at 68°C. Amplified PCR products were stored at a temperature of 4°C until they were needed for analysis. Products were separated using the ABI 3130 Genetic Analyzer (Applied Biosystems) with Genescan™ 500 LIZ™ as the size standard. Samples were genotyped with GeneMapper (version 4.0; Applied Biosystems).

Table 2. Locus, primer sequence, fluorescent label, and SSR motif for the 6 microsatellite loci in *A. triloba*.

Locus	Primer sequence	Label	Enriched motif
Pp-B3	Forward: GCGAAAACGAACATACCTC Reverse: CCTCCTCCACCACCACTAC	FAM	GA
Pp-B103	Forward: ATGCCCCAACAGAGACTTC Reverse: GGATGAGACACTCGGCTTAC	FAM	GA
Pp-B117	Forward: GCATTGGTGTTTAGAACTTCTC Reverse: GCACAACAAAAAAGGATAAGAC	HEX	GA
Pp-G103	Forward: AGCCAAAATCAAGAAACC Reverse: CTGCTCAGGGTCACTACA	FAM	AAT
Pp-G119	Forward: AAACCGTAGTAAAACCAGACAA Reverse: GGATAGGAAAACATGGTGATTA	FAM	ATT
Pp-G124	Forward: GTAGCCAGGAGGAGATGAACT Reverse: GGTGATTGGATTGCCTAAAAT	HEX	AAT

DNA Analysis

PowerMarker (version 3.25, Lui and Muse 2005) software was used to calculate the following statistics: major allele frequency (most common allele), number of genotypes, observed number of alleles (n_A), expected heterozygosity (H_e, expected number of heterozygous genotypes at a given locus divided by the number of genotypes at the locus), and polymorphism information content (PIC, an estimate that the parental origin of an allele can be determined from the marker locus genotype in any given offspring).

Pawpaw and Invasive Plant Incidence

Each *A. triloba* population was measured for its length and width. Three 5 × 5 m (25 m^2) plots (left, right, and middle) were outlined and the numbers of stems of *A. triloba* and *L. maackii* were counted. Control plots (5 m^2) were marked upstream/downstream of similar habitat to the patch and *A. triloba* and *L. maackii* stems were counted. Additional invasive plant species [garlic mustard (*Alliaria petiolata*), winter creeper (*Euonymus fortunei*), and colts foot (*Tussilago farfara*)] were also counted if found in the *A. triloba* or *L. maackii* plots.

Plant Stem Analysis

The average of the number of stems of each species (pawpaw, honeysuckle, garlic mustard, and winter creeper) in each plot was calculated. Averages were entered into JMP Statistical software (9.0.0 ©2010 SAS Institute Inc.) and a matched pairs analysis was run to determine effect of pawpaw on invasive plants. Data were analyzed using a paired *t*-test with a level of significance of 0.05.

RESULTS

Asimina triloba Genetics

The pawpaw populations examined displayed high levels of genetic diversity based on the SSR markers amplified. Of the six loci examined (Pp-B3, Pp-B103, Pp-B117, Pp-G103, Pp-G119, Pp-G124), all yielded products that could be used for analysis (Table 3). Of the 140 individual pawpaw trees sampled, and 6 loci examined, there were 51 genotypes and a total of 45 alleles amplified. There was great variation for each locus in terms of the

Table 3. Major allele frequency, genotype number, allele number, allele size (base pairs), gene diversity, heterozygosity, polymorphism information content (PIC), and SSR motif for all patches by locus.

Marker	Major allele frequency	Genotype No	Allele No	Allele size (bp)	Gene diversity	Heterozygosity	PIC	Motif
Pp-G103	0.60	8.0	5.0	270–298	0.59	0.40	0.55	AAT
Pp-G119	0.37	6.0	4.0	158–167	0.70	0.91	0.64	AAT
Pp-B117	0.46	6.0	5.0	110–179	0.68	0.64	0.63	GA
Pp-B3	0.21	8.0	8.0	175–195	0.85	0.86	0.83	GA
Pp-B103	0.18	16.0	18.0	264–347	0.89	0.74	0.87	GA
Pp-G124	0.47	7.0	5.0	192–204	0.63	0.38	0.56	AAT
Mean	0.38	8.5	7.5	110–347	0.72	0.65	0.68	

number of genotypes (6–16), number of alleles (4–18), the major allele frequency (0.18–0.60), and the allele size (110–347).

All genotypes were separated using a UPGMA dendrogram, depicting the relationship between area, patch, and individual sampled (Figure 1). The dendrogram shows that all patches displayed at least two genotypes in each patch. After examining six loci, all of the *A. triloba* patches exhibited some degree of clonality to some degree ranging from two – thirteen genotypes. Because all of the patches sampled contained at least two genotypes, none were completely clonal. The patches KYR G and KSU F displayed only two genotypes per patch, respectively, indicating a high degree of clonality in these patches across the patch transect that was sampled. The dendrogram showed that *A. triloba* had a high genetic diversity between patches; based on the dendrogram break points, the patches are quite genetically diverse from each other. Cove Spring Park patch (CSP) B was of great interest. The CSP B patch was the most highly differentiated, containing thirteen genotypes and falling into two separate locations on the dendrogram. Conversely Kentucky State University Research and Demonstration Farm patch KSU F and Kentucky River patch KYR G only contained two genotypes. The Environmental Education Center (EEC) C was the most genetically dissimilar, and EEC D and KSU F exhibited the most clonality of any of the other patches. Geography of the patches seems to have little to no effect on genetic similarity.

Pawpaw and Invasive Plant Incidence

A total of three different invasive species [garlic mustard (*Alliaria petiolata*), Amur honeysuckle, (*Lonicera maackii*), and winter creeper (*Euonymus fortunei*)] were found in many of the plots sampled (Tables 5, 6). Unfortunately, pawpaw patches and neighboring control plots that were examined at the EEC locations did not contain honeysuckle plants and were not useful for this portion of the study; this area has so far escaped honeysuckle infestation. Due to similarity in niche *L. maackii* was of greatest interest to the study. None of the invasive plant densities were statistically different in the pawpaw plots from the control plots sampled. Three patches appeared to have fewer honeysuckle plants inside each pawpaw patch than outside the patch. A fourth patch had similar numbers of honeysuckle within and outside the patch. However, there were not significantly fewer honeysuckle plants in pawpaw patches.

There was a strong trend for pawpaw patches to contain larger populations of garlic mustard than control plots (P-value > 0.0512; Table 5). There was not a significant difference between winter creeper incidences in pawpaw patches; however there appeared to be more plants inside patches KSU E and KSU F.

DISCUSSION

Genetics

This is the first report of the assessment of genetic diversity and clonality of native pawpaw populations using the SSR methodology. *Asimina triloba* is a plant species that is known to produce rootsuckers. In terms of genetic diversity, the expected heterozygosity H_e (0.65) was lower than reported in Pomper et al. 2010 for cultivated pawpaw varieties, but similar to results published by Lu et al. 2011 and polymorphism information content PIC (0.68) was equitable to both published results. Only two nucleotide repeat motifs were chosen for the experiment, GA (loci Pp-B3, Pp-B103, and Pp-B117) and AAT (loci Pp-G103, Pp-G119, Pp-G124). The GA motif had a major allele frequency of (0.29), number of genotypes (10.0) and H_e of (0.75). The AAT motif had a major allele frequency of (0.48), number of genotypes (7.0), and H_e of (0.56). Based on the loci examined in the study, none of the patches were completely clonal. Alleles per locus was 7.5 in the pawpaw patches as compared to other cultivated crops sampled such as 13.3 for hazelnut (Bassil et al. 2005) 12.1 for apple (Hokanson et al. 1998, 2001) and 9.1 for pear (Sisko 2009.). When comparing the results from this experiment to known data from Pomper et al. (2010) and Lu et al. (2011) the data was comparable. Ranges from their studies were: major allele frequency 0.38–0.48, genotype number 10.8–20, allele number 7.3–12, allele size 144–343, heterozygosity 0.66–0.7, and PIC 0.63–0.68. Results from this experiment were the following: major allele frequency 0.38, genotype number

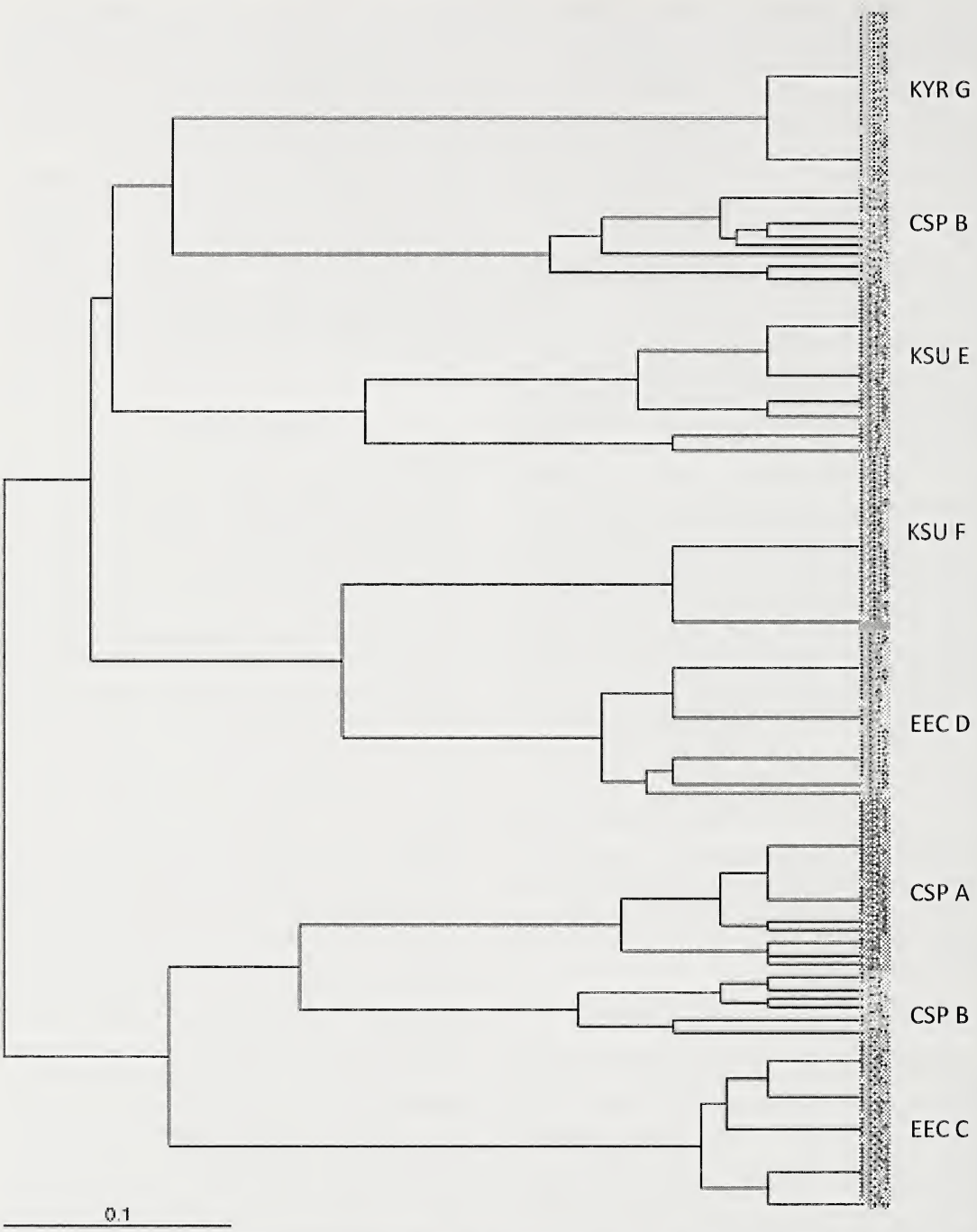

Figure 1. UPGMA dendrogram generated showing all individuals sampled and highlighted to demark its respective patch.

8.5, allele number 7.5, allele size 110–347, heterozygosity 0.65, and PIC 0.68. All patches showed clonality to some degree and KSU F and KYR G only showed 2 genotypes for all 20 stems sampled. When comparing Table 4 to Figure 1 it becomes clear that EEC C had the lower density of pawpaw stems per plot (11), with a similar number of pawpaw stems in the

Table 4. Pawpaw stem density, patch length, and number of genotypes found by patch.

Patch	Pawpaw stem density (stems/m²)	Patch length (ft)	# of Genotypes
CSP A	0.85	72	7
CSP B	0.95	183	13
EEC C	0.44	82	5
EEC D	0.87	116	5
KSU E	0.99	103	6
KSU F	0.99	75	2
KYR G	0.65	70	2

other plots. Based on the data gathered from the experiment there is not a statistically significant relationship between A. triloba genetic diversity and any of the factors examined.

Invasive Species Plots

From the data obtained, it is difficult to determine if there is a negative relationship between the presence of *A. triloba* and *L. maackii*. While KSU F and CSP A showed a trend that would support the hypothesis, the low incidence of honeysuckle inside and outside four of the patches do not provide enough additional information to reject or accept our hypothesis. Patch KSU F was highly clonal and CSP A did have a large clonal portion of the patch. KYR G was highly clonal, but not statistically different from the control to support the hypothesis that presence of *A. triloba* will have a negative impact on *L. maackii*. Additional patches at different locations and higher honeysuckle pressure will be required to further test our hypothesis. It was interesting that garlic mustard had higher incidence in most pawpaw patches; protection by patches could be important in supporting the life cycle of this plant. No correlation could be identified between density of pawpaw stems, number of genotypes per patch or patch length (Table 4).

Other variables could also be important to the incidence of invasive plants in pawpaw patches. All patches except KYR G, which grew beside the Kentucky River, were located along stream banks. All the patches except the EEC patches (not seen in the table) were located on a steep bank; however KYR G and CSP A were much steeper than the others. All patches were found in the forest understory, although no light level readings were recorded during the progression of the season or during patch sampling to determine if light could have been a contributing factor. Just the presence of *A. triloba* was not enough to deter *L. maackii* occurrence. Additional factors such as soil, light, and *A. triloba* canopy shading need to be considered when determining correlations between *A. triloba* and *L. maackii*.

Within the parameters of the experiment only stem numbers were counted, however there may be other factors that may help one understand the relationship. Examining stem diameter, stem height, leaf biomass, soil samples, light levels, water availability, etc. may reveal additional variables that influence pawpaw patch density and invasive plant incidence. Since *L. maackii*'s foliage is present earlier in the spring and later into the autumn compared to *A. triloba*, it should be investigated whether periods of high light penetration are able to sustain *L. maackii* throughout the season. Also, *L. maackii* may use allelopathy, therefore its effects on *A. triloba* should be examined. It is worth noting that CSP B contained colt's foot in higher density than the control, but this was the only patch containing the species.

In conclusion, the pawpaw populations in Kentucky examined displayed high levels of genetic diversity based on the DAN microsatellite markers amplified. All patches showed clonality to some degree in transects of patches. Three patches appeared to have fewer honeysuckle plants inside each pawpaw patch than outside the patch. A fourth patch had similar numbers of honeysuckle within

Table 5. Invasive plant population, by species, in plots with, and without pawpaws.[1]

Treatment	Count (plants/plot) ± SE		
	Garlic mustard	Honeysuckle	Winter creeper
Control	798.3 ± 230.7	8.5 ± 3.1	18.5 ± 9.8
Pawpaw	1123.8 ± 219.0	3.6 ± 1.3	19.3 ± 10.9

[1] No significant difference detected between treatments.

Table 6. Number of Pawpaws, Honeysuckle, Garlic mustard, and Winter creeper by treatment and location.

Location	Treatment	Pawpaw	Honeysuckle	Garlic mustard	Winter creeper
CSP A	Control	0.0	16.3	2750.0	0.0
	Pawpaw	21.3	1.7	3083.3	0.0
CSP B	Control	0.0	0.0	125.0	6.7
	Pawpaw	23.7	1.7	250.0	0.0
EEC C	Control	0.0	0.0	541.7	0.0
	Pawpaw	15.7	0.0	391.7	0.0
EEC D	Control	0.0	0.0	105.0	0.0
	Pawpaw	21.6	0.0	93.3	0.0
KSU E	Control	0.0	6.3	666.7	16.7
	Pawpaw	24.7	11.0	1583.3	33.3
KSU F	Control	0.0	34.7	291.7	6.3
	Pawpaw	24.7	11.0	750.0	10.4
KYR G	Control	0.0	2.0	1108.3	100.0
	Pawpaw	16.3	0.0	875.0	91.7

and outside the patch. However, there were not fewer honeysuckle plants in pawpaw patches than control plots. Additional patches at different locations and higher honey suckle pressure and possibly different sampling approaches will be required to further test whether pawpaw patches reduce the incidence of honeysuckle bushes.

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Abstracts of Some Papers Presented at the 2010 Annual Meeting of the Kentucky Academy of Science

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AGRICULTURE

Evaluation of Sunflower (*Helianthus annuus* L.) Cultivars for Cut Flowers. CHRISTOPHER G. FERGUSON MARTIN J. STONE and ELMER GRAY*, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The sunflower (*Helianthus annuus* L.), an ancient cultivated crop for oilseed production, more recently has gained acceptance as a cut flower in the florist industry. Objectives of the present study were to survey available sunflower cultivars for adaptability to Kentucky growing conditions and for suitability to local florist markets. In 2011, sunflower cultivar trials were conducted at Bowling Green (36.93 N, 86.47 W) and Owensboro (37.78 N, 87.14 W) Kentucky. The randomized complete block design included 18 diverse cultivars and three replications. An experimental unit consisted of 10 plants of each cultivar spaced 30 × 60 cm. apart. Data were collected on seedling emergence, days to flowering, plant height, head diameter, stem diameter, branching, petal color, pollen production, and vase life. Twice weekly harvests of flowering heads began 29 July and continued through mid-September resulting in 9 and 10 harvests in Bowling Green and Owensboro; respectively. Flowering heads were evaluated by both lay and professional groups. The data on plant and head characteristics are being stratified to determine the most adapted cultivars for growing in Kentucky and the ones most acceptable by the florist industry.

Effect of Tassel Removal for Baby Corn Production in Kentucky. CHRISTOPHER G. FERGUSON*, ZHENG WANG, MARTIN STONE, and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101-3576.

Baby corn (*Zea mays* L.) consists of unfertilized young ears harvested at silk emergence. The 2011 study was a culmination of four successive years of production and evaluation of baby corn at Western Kentucky University (36.93 N, 86.47 W). The objective was to compare the effect of tassel removal on baby corn (BC) production on four cultivars, two field ('N77P-3000GT', 'N68B-3000GT') and two sweet ('Silver Queen', 'Peaches N Cream') corns. Results indicated that tassel removal gave significant increases ($P < 0.01$) of BC ears across harvests (H) and cultivars; however, the effect was not consistent over treatments. For harvests, the difference due to detasseling was highly significant ($P < 0.01$) for H1, significant ($P < 0.05$) for H2 and H3, but not significant ($P > 0.05$) for H4. For cultivars, numerical values were higher for detasseled than non-detasseled treatments in the first three harvests for each cultivar, but significant ($P < 0.05$) only for Peaches N Cream. Quality of BC from both tassel treatments decreased in H3 and H4. Further study is needed to determine economic returns of detasseling BC.

Developing a Biofilter for Remediation of Pesticide Residues in Runoff Water. GEORGE ANTONIOUS, College of Agriculture, Food Science, and Sustainable Systems, Department of Plant and Soil Sciences, Kentucky State University, Frankfort, KY 40601.

Bioremediation is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms. Nine biobeds (ground cavity filled with a mixture of composted organic matter, topsoil, and a surface grass) were established at Kentucky State University research farm (Franklin County, KY) to study the impact of this practice on reducing surface runoff water contamination by residues of dimethazone and trifluralin herbicides arising from an agricultural field. Biofilter systems were installed at the bottom of the slope of specially designed runoff plots to examine herbicides retention and degradation before entering streams and rivers. In addition to biofilter systems, three soil management practices: i) municipal sewage sludge (SS), ii) SS mixed with yard waste compost (SS + YW), and iii) no-mulch rototilled bare soil (NM used for comparison purposes) were used to monitor the impact of soil amendments on herbicide residues in soil following natural rainfall events. Organic amendments increased soil organic matter content and herbicide residues retained in soil following rainfall events. Biofilters installed in NM soil reduced dimethazone and trifluralin by 84 and 82%, respectively, in runoff water that would have been transported down the land slope of agricultural fields and contaminate natural water resources. Biobeds installed in SS and SS + YW treatments reduced dimethazone by 65 and 46% and trifluralin by 52 and 79%, respectively. The use of biofilters in on-farm bioremediation of pesticide residues in surface runoff water might provide a potential solution to contaminated runoff and seepage water arising from agricultural production operations.

Evaluation of Constructed Wetlands for Nitrate and Phosphorus Removal. ERIC T. TURLEY* and GEORGE F. ANTONIOUS, College of Agriculture, Food Science, and Sustainable Systems, Department of Plant and Soil Sciences, Kentucky State University, Frankfort, KY 40601.

Constructed wetlands are designed and utilized to reduce or eliminate the effect of agrochemicals on water quality. The use of agrochemicals, such as fertilizers, requires practices for remediation of these environmental contaminants. At Kentucky State University Research Farm, twelve constructed wetland microcosms were

established. Six microcosms were filled with river gravel and six with grade #2 limestone. Sweet flag plants, *Acorus calamus*, were planted in six microcosms (nine plants in three river gravel microcosms and nine plants in three limestone microcosms). The plants were observed for growth and performance in the two types of rocks. Water samples were collected at regular time intervals to monitor the performance of the microcosms. Improvement was noted in orthophosphate, nitrate, ammonia, and dissolved oxygen concentrations. The results revealed that microcosms containing limestone, with and without sweet flag, had a 23% reduction of orthophosphate ions compared to microcosms with river gravel with and without sweet flag. The NO_3-N content in microcosms containing limestone and planted with sweet flag was reduced by 42% compared to microcosms containing river gravel and planted with sweet flag. Microcosms containing either limestone or river gravel and sweet flag had increased dissolved oxygen content over microcosms containing either limestone or river gravel and no plants. The NH_3-N content in all microcosms was reduced to near immeasurable amounts.

Half-lives of Endosulfan Isomers on Field Treated Vegetables. KYLA ROSS*[1], GEORGE ANTONIOUS[1], and TEJINDER KOCHHAR[2], [1]College of Agriculture, Food Science, and Sustainable Systems, and [2]Department of Biology, Carver Hall, Kentucky State University, Frankfort, KY 40601.

Endosulfan 3 EC, a mixture of α- and β-stereo isomers, was sprayed on field-grown pepper and melon plants at the recommended rate of 0.44 kg A.I. $acre^{-1}$. Plant tissue samples (leaves and fruits) were collected 1 h to 30 days following spraying and analyzed for endosulfan isomers. Analysis of samples was accomplished using a gas chromatograph (GC) equipped with a mass detector in total ion mode. The results indicated the formation of endosulfan sulfate as the major metabolite of endosulfan sulfite and the relatively higher persistence of the β-isomer as compared to the α-isomer. The initial total residues (α- and β-isomers plus endosulfan sulfate) were higher on leaves than on fruits. On pepper and melon fruits, the α-isomer, which is the more toxic to mammals, dissipated faster ($T_{1/2}$ = 1.22 and 0.95 d, respectively) than the less toxic β-isomer ($T_{1/2}$ = 3.0 and 2.5 d, respectively). These results confirm the greater loss of the α-isomer compared to the β-isomer, which can ultimately impact endosulfan dissipation in the environment. The higher initial residues of endosulfan on pepper leaves should be considered of great importance for timing field operations and the safe entry of harvesters due to the high mammalian toxicity of endosulfan.

Antioxidants and Heavy Metals Content of Hot Pepper. MCKENZIE JOHNSON*[1], GEORGE ANTONIOUS[1], and TEJINDER KOCHHAR[2], [1]College of Agriculture, Food Science, and Sustainable Systems, and [2]Department of Biology, Carver Hall, Kentucky State University, Frankfort, KY 40601.

Hot pepper accessions that strongly accumulate heavy metals in their edible portions should be regarded as potential source of heavy metal contamination in the food supply. Phenols, ascorbic acid, capsaicin, and β-carotene are some of the classes of naturally occurring compounds having antioxidants activity in hot pepper. However, elevated concentration of heavy metals in hot pepper fruits could expose consumers to potentially hazardous chemicals. The main objectives of this investigation were to: i) to select candidate accessions of hot pepper having high concentrations of phytochemicals for use as parents in breeding for these antioxidant compounds, and ii) assess if hot pepper genotypes that contain great concentrations of capsaicin are also heavy metals (Cd, Cr, Ni, Pb, Zn, Cu, Mo) accumulators. Seeds of hot pepper (*Capsicum chinense*) were collected from Belize, Brazil, Colombia, Ecuador, Mexico, Peru, Puerto Rico, and U.S. and planted in a silty-loam soil. Fruits of PI-640900 (USA) contained the greatest concentration of capsaicin (1.52 mg g^{-1} fruit) and dihydrocapsaicin (1.16 mg g^{-1} fresh fruit), while total major capsaicinoids (capsaicin and dihydrocapsaicin) in the fruits of PI-438648 (Mexico) averaged 2 mg g^{-1} fruit. PI-152452 (Brazil) and PI-360726 (Ecuador) contained the greatest concentrations of ascorbic acid (1.2 and 1.1 mg g^{-1} fruit, respectively). While PI-438648 (Mexico) contained the greatest concentration of total phenols contents (349 μg g^{-1} fruit), PI-355817 (Ecuador) contained the greatest concentration of β-carotene among the other 63 accessions tested. Variability of these traits might be utilized via plant breeding approaches for their value-added health-promoting characteristics.

An Update on the KSU Pawpaw Breeding Program. KIRK W. POMPER*, SHERI B. CRABTREE, and JEREMIAH D. LOWE, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355.

The North American pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit native to the eastern United States, which is in the early stages of domestication. Pawpaw fruit have fresh market appeal for farmers' markets, community supported agriculture, and organic markets, as well as processing potential for frozen pulp production. New high yielding cultivars with excellent fruit quality would assist in the development of a pawpaw industry. Kentucky State University (KSU) serves as the National Clonal Germplasm Repository for pawpaw, and germplasm evaluation is an important research priority. Pawpaw germplasm has been screened for superior fruiting characteristics and trial as new pawpaw varieties. Pawpaw genotypes in the KSU repository orchards under evaluation are from crosses of current pawpaw varieties or are open pollinated seedlings from a range of genetic sources. Some selections that produced high yields and excellent fruit quality have been selected for clonal propagation (budding onto rootstock) and field trials. Selections that have shown excellent fruit quality and yields include Hi4-1, Hi7-5, H3-120, G4-21, G4-25, G5-23, G6-120, G9-109, and G9-111. Most of the pawpaw advanced selections had similar

budding success and vigor to controls and are in field trials. When the genetic diversity of KSU advanced selections were evaluated using Simple Sequence Repeat DNA markers, these selections displayed significant genetic diversity compared to pawpaw cultivars recently released by the PawPaw Foundation breeding effort. KSU advanced selections contain unique pawpaw germplasm that should enhance the genetic base of cultivars if these selections are released to the public.

Ethanol Production Potential from Pawpaw Fruit Agricultural Waste. BRANDON K. MAY*, MICHAEL BOMFORD, KIRK W. POMPER, JON CAMBRON, and TONY SILVERNAIL, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355.

The finite nature and rate of depletion of fossil fuels has prompted discussion into the production of biofuels to supplement our national energy demands. Government mandates diverting corn or other grain products into cellulosic ethanol production has caused speculation into consequences of a reduced grain supply leading to increased food and livestock costs. Globally, studies are also being conducted to examine the potential useable sugars and starches derived from agricultural waste products ranging from olive mill waste, corn stover, peanut, and fruit waste. The pawpaw [*Asimina triloba* (L.) Dunal] is a native tree fruit and is a new high-value fruit crop in Kentucky. Pawpaw fruit have fresh market appeal for farmers' markets, community supported agriculture, and organic markets, as well as processing potential for frozen pulp production. After harvesting of the pawpaw fruit many poorer quality fruit remain unused in the orchard. Seed is a byproduct of pulp extraction of the poor quality fruit; the seed is valuable to nurseries, often selling for $10 per pound. Pulp waste from seed extraction has potential for ethanol production. In initial experiments with pawpaw pulp waste from seed extraction, the extracted pulp sugar contents were found to be as high as 19° Brix or 112.3 g/L and once processed would yield 10.3% alcohol by volume. Using methods devised by UC Davis for sugar to ethanol conversion, biofuel production potential of the pawpaw fruit appears promising from this agricultural waste. The potential for ethanol production and extraction methods for pawpaw pulp will be discussed.

Prime-Ark®45 and Prime-Jan® Primocane Fruiting Blackberry Production Grown Under Organic Culture in Kentucky. JEREMIAH D. LOWE*[1], KIRK W. POMPER[1], SHERI B. CRABTREE[1], JOHN R. CLARK[2], and JOHN G. STRANG[3], [1]College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355, [2]Fruit Culture & Breeding, 316 Plant Science Bldg. University of Arkansas, Fayetteville, AR 72701, and [3]Department of Horticulture, N-318 Agricultural Sciences North, University of Kentucky, Lexington, KY 40546.

Primocane-fruiting blackberries are attractive to Kentucky growers because they can be grown organically and have the potential to produce a niche-market crop from late summer until frost. Therefore, locally produced fruit from primocane blackberry selections can be harvested from July until usually October, providing fruit for sale at farmers' markets, community supported agriculture, and organic markets. In June 2010, a blackberry trial was planted at the KSU Research and Demonstration Farm on the certified organic land. The planting contained four replicate blocks each of the selections of Prime-Jan®, a primocane-fruiting selection from the University of Arkansas, and Prime-Ark®45, a newly released primocane-fruiting blackberry. Plants were arranged in a completely randomized design, with four replicate plots each containing five plants of each selection or cultivar (total of 20 plants of each selection or cultivar) in 10-foot plots. This trial was managed with organic practices following the National Organic Program standards. A combination of cultivation, hand weeding, and straw mulch was used for weed control. Drip irrigation was used as needed. Ripe fruit were harvested from the plants twice weekly, Monday and Thursday, from July until October 2011. Harvest period, yield, and berry weight were recorded for all selections. Prime-Ark®45 had higher yield and greater berry size as compared to Prime-Jan®. Warm summer temperatures in 2011 appeared to reduced fruit set on Prime-Jan® as compared to Prime-Ark®45. Prime-Ark®45 appears to be a desirable cultivar well suited to Kentucky growing conditions.

Kentucky State University Pawpaw Processing: An Update. SHERI B. CRABTREE*, KIRK W. POMPER, and JEREMIAH D. LOWE, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601.

The pawpaw (*Asimina triloba*) is the largest tree fruit native to the United States and is being grown on a small scale commercially in Kentucky and surrounding states. Pawpaws produce unique fruit with creamy yellow-orange flesh and a flavor resembling a blend of mango, banana, and pineapple. The fruit's short shelf life and difficulty with postharvest handling and storage are impediments to commercial development. Marketing frozen fruit pulp as a value-added product could be one solution to this problem. However, pawpaw fruit pulp extraction is labor-intensive and made more difficult by the row of large inedible seeds in the fruit, and valuable pulp may be lost through ineffective extraction methods. The objective of this study was to determine if processed pulp recovery rate differs by pawpaw cultivar. Three sets of five ripe fruit each of five pawpaw cultivars (KSU-Atwood, Mitchell, PA-Golden, Sunflower, and Susquehanna) were selected. Fruit were processed using a modified Roma Sauce Maker with a grape spiral and squash/pumpkin screen to separate seeds from pulp and macerate the pulp. Individual fruit and seed weights were measured to determine percent seed. Processed pulp recovery rate varied significantly among cultivars, with Susquehanna, KSU-Atwood, and Sunflower having a greater percentage

of pulp recovered from fruit than Mitchell. Susquehanna, KSU-Atwood, and Sunflower had higher fruit weights than PA-Golden or Mitchell. With large fruit weights, a high rate of processed pulp recovery, and high fruit: seed ratio for more efficient processing, the cultivars KSU-Atwood, Susquehanna, and Sunflower are good choices for Kentucky pawpaw growers.

Beneficial Insects Associated with Fall Established Native Perennial Plant Borders. JERMAINE DUNIGAN*, JOHN D. SEDLACEK, and KAREN L. FRILEY, Kentucky State University, Frankfort, KY 40601.

Native perennial plants are ideal for use in sustainable landscapes and are beneficial to the environment because they create habitat for wildlife species including beneficial insects. Attracting beneficial insects using a farmscaping approach can be important in trying to establish sustainable methods of insect pest management in vegetable and fruit crops. Some researchers have suggested that non-crop vegetation such as grasses and floral strips planted in crop field margins can enhance predaceous arthropod and parasitoid populations. Therefore, the objective of this study was to compare several insect groups colonizing newly established perennial borders vs. non-mowed mixed grass/weedy pasture borders. This study was conducted at the Kentucky State University Research and Demonstration Farm in Franklin County, KY. Sixteen plant species, including big bluestem (*Andropogon gerardii*), thimbleweed (*Anemone virginiana*), New England aster (*Aster novae-anglica*), side-oats grama (*Bouteloua curtipendula*), purple coneflower (*Echinacea purpurea*), gray-headed coneflower (*Ratibida pinnata*), rattlesnake master (*Erygium yuccifolium*), common boneset (*Eupatorium perfoliatum*), blue lobelia (*Lobelia siphilitica*), bee balm (*Monarda fistulosa*), switchgrass (*Panicum virgatum*), foxglove beardtongue (*Penstemon digitalis*), hairy beardtongue (*Penstemon hirsutus*), slender mountain mint (*Pycantheum tennuifolium*), little bluestem (*Schizacharium scoparium*), and prairie dropseed (*Sporobolus heterolepis*), were established in 25 m × 2 m border rows replicated three times. Insects were sampled using four 15 cm × 15 cm sticky traps mounted to tobacco sticks in each border row. Lady beetles, solitary bees in the genus *Agapostemon*, soldier beetles (*Chauliognathus pensylvanicus*), and green lacewings (*Chrysops* sp.) were caught in higher numbers in the native perennial border rows than the pasture borders.

Will Mowing of Primocane-fruiting Blackberries Affect Fruit Ripening? KAREN L. FRILEY*, JOHN D. SEDLACEK, KIRK W. POMPER, JEREMIAH D. LOWE, MICHAEL K. BOMFORD, SHERI B. CRABTREE, MARQUITA L. GRAYSON-HOLT, CHRISTOPHER M. WALES, and RACHEL S. HAYDEN, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Blackberry acreage has increased in Kentucky, while demand still often exceeds supply. The need for sustainable production practices is important for small and limited resource farmers as well as organic producers. Primocane-fruiting blackberries will set on both the overwintered canes as well as the current season primocanes. These varieties will produce two crops per pear – the regular summer crop on the floricanes and then a later crop on the primocanes. 'Prime Jim®' and 'Prime Jan®', which are two commercially available primocane-fruiting varieties, were used in this study. Three replicates of each of the two varieties were initially mowed to ground level 6 April, while the second mowing occurred on 24 June. Fruit ripening in Prime Jim plots began to drop off in late August, while fruit ripening remained constant throughout the season in Prime Jan plots.

Stink Bug Species in Organic Blackberries. MARQUITA L. GRAYSON-HOLT*, JOHN D. SEDLACEK, KAREN L. FRILEY, KIRK W. POMPER, JEREMIAH D. LOWE, MICHAEL K. BOMFORD, SHERI B. CRABTREE, CHRISTOPHER M. WALES, and RACHEL S. HAYDEN, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Stink bugs (Hemiptera: Pentatomidae) are pests of blackberries in Kentucky. These insects insert their beak into drupelets to extract the juice and may also leave a foul odor and taste. Consumer demand for damage-free produce means that growers must use safe and effective management tactics for insect pests. Organic blackberry growers require sustainable and environmentally sound production methods to manage these insects. Spring-mowing of primocanes, on primocane fruiting blackberry varieties could avoid stink bug attack and delay fruit set. In 2011, three replicate plots of each of two varieties, 'Prime-Jim®' or 'Prime Jan®' were initially mowed to ground level on 6 April. Three replicate plots of each variety were then mowed a second time on 24 June. Stink bugs were sampled weekly using Florida Stink Bug Traps and hand collection methods. Stink bugs were found across treatments during the 2011 sampling period which extended from 11 July until 29 September. Five stink bug species were identified during the period of fruit ripening in the planting. The green stink bug was the most abundant, followed by rice stink bug and then brown, twice stabbed and one-spotted stink bugs at 53%, 16%, 11%, 11% and 11%, respectively. Both hand collection of stink bugs and the use of the Florida Stink Bug Traps resulted in the capture of stink bugs. Although hand collecting required more time, more than twice as many stink bugs were captured compared to the stink bug trap, at 68% and 32%, respectively.

Does a Methyl Salicylate-based Lure Attract Lady Beetles to Blackberries? JUSTINA RIDDICK*, JOHN D. SEDLACEK, KAREN L. FRILEY, and JOY BIRIKE, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Kentucky produces approximately 45 ha of blackberries for a total value of $1,000,000 annually. Demand for locally grown and damage-free blackberries usually

exceeds the supply. Developing more sustainable production methods, including the use of beneficial insect attractants, such as a methyl salicylate-based lure, is important for the success of small and limited resource farmers. Eight blackberry plots, including six collaborators, were located in Franklin, Fayette, Scott and Shelby counties in Kentucky. Three plots were certified organic and the other five plots had no pesticides applied. Four sticky traps and posts were placed in all plots and two PredaLure® lures were placed in each of the PredaLure plots. Sticky traps were collected weekly for twelve weeks, placed in labeled ziplock bags and taken to the laboratory where lady beetles were identified using an illuminated magnifier. Total number per species and average number per trap were then calculated. Pink lady beetle, *Coleomegilla maculata*; seven-spotted lady beetle, *Coccinella septempunctata*; Asian lady beetle, *Harmonia axyridis*; parenthesis lady beetle, *Hippodamia parenthesis*; spotless lady beetle, *Cycloneda munda*; orange-spotted lady beetle, *Brachicantha ursina*; twice-stabbed lady beetle, *Chilocoris stigma*; and mildew eating lady beetle, *Psyllobora vigitimaculata* were caught in PredaLure baited sites. Parenthesis and seven-spotted lady beetles were not caught in non baited sites. PredaLure plots had more pink lady beetles, while non PredaLure plots had more Asian, spotless, and mildew-eating lady beetles.

Lady Beetles Associated with Sweet Corn Bordered by Pasture, Buckwheat or Sunflower Borders. JOHN D. SEDLACEK*, KAREN L. FRILEY, MARQUITA L. GRAYSON-HOLT, CHRISTOPHER M. WALES, and RACHEL S. HAYDEN, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Sweet corn, *Zea mays* 'Garrison®', was grown in replicated plots on Kentucky State University's Agricultural Research and Demonstration Farm in Franklin County, KY. Each 25 m × 12 m plot was bordered on each side of its length by a 2 m wide border of unmowed pasture, buckwheat (*Fagopyrum esculentum*), or dwarf sunflower (*Heliathus annuus* var. Big Smile). A randomized complete block design replicating each treatment five times was used and all plots were separated by 25 m. Yellow sticky traps (15 cm × 15 cm) were used to capture lady beetles. Two traps were deployed at canopy height between the edges and equidistant from the ends of each border. Four traps were deployed in each sweet corn plot, one in the center of each plot quadrant. Traps were changed weekly through anthesis. Sticky traps were placed individually in ziplock plastic bags, labeled, and transported to the laboratory for insect identification and enumeration. Pink lady beetle, *Coleomegilla maculata*; Asian lady beetle, *Harmonia axyridis*; spotless lady beetle, *Cycloneda munda*; and seven spotted lady beetle, *Coccinella septempunctata* were caught in this study. The pink lady beetle was the most abundant species in all three border types and the sweet corn plots with 79% and 94% of the lady beetles caught in the borders and sweet corn, respectively. Pink lady beetle numbers decreased in buckwheat from 14 August through 27 August, but increased markedly in sweet corn from 14 August to 20 August, potentially indicating movement into the sweet corn.

Economics Systems, Role of Government, and Agriculture. STEPHEN A. KING, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101, Stephen.King2@wku.edu.

The role of government in U.S. agriculture is in great part defined by the Farm Bill. The current U.S. Farm Bill is referred to as the "Food, Conservation, and Energy Act of 2008" and its provisions have far reaching impacts over a broad range of constituents. It is set to expire in the year 2012, at which time a new Farm Bill is expected to be enacted. In addition to traditional commodity programs that directly impact the decisions of farmers, the legislation impacts the development of organic agriculture and biofuels markets, conservation of natural resources, nutrition and rural housing programs, agricultural research, among other areas. As a society, how do we decide what should and should not be included in the Farm Bill? Do we have any set of criteria for deciding the role of government in agriculture? How does current and past farm legislation influence the economic system of the U.S.? The work presented addressed these questions. It has been hypothesized that our socio-economic values influence the political process and thus the legislation that is developed and in turn the role of government in agriculture and therefore the economic system. In general the research suggests that current and past agricultural legislation moves our economic system toward the direction of centrally planned capitalism, that is agricultural resources are predominately owned by the private sector but their allocation is strongly influence by governmental policy. A set of criteria were proposed for guiding the role of government in agriculture.

Characteristics of and Reasons Why Farmers Choose Off-farm Work. CAITLIN N. CARTER, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101. caitlin.carter472@topper.wku.edu.

In recent years, farmers in the United States have been seeking off-farm work to supplement farm operations income. In many cases, income provided by off-farm work is the largest component of the farm household income. Previous research by the United States Department of Agriculture – Economic Research Service suggests that the extent to which producers rely on off-farm income is dependent upon farm size and the type of enterprise. Results of this study reveal the most prominent characteristics of those farmers who choose off-farm work, the reasons why they choose off-farm work, and the extent of income that off-farm work provides to various categories of farm households.

ANTHROPOLOGY AND SOCIOLOGY

Acculturation and Body Weight Status of Chinese Immigrants in Kentucky. CECIL BUTLER*, LINGYU HUANG, and CHANGZHENG WANG, Human Nutrition Program, Kentucky State University, Frankfort, KY 40601.

Traditional Chinese diets are rich in vegetables and fruits and obesity is less prevalent among Chinese people.

Immigrants adapt to American diets and behavior patterns. The objective of this study was to assess the acculturation and body weight status of Chinese immigrants in Kentucky. Thirty Chinese American immigrants were recruited to participate in the study at a large community event. The subjects were asked to fill out a questionnaire before they were given a free analysis of their body composition (body fat %) with a Tanita TBF-521 body composition analyzer. Body mass indexes were calculated from the body weight and height measured on-site. 84% of the participants were within normal body weight range with only 10.5% in the overweight and 5% in the obese category. Close to 50% of them speak Chinese and English about the same, but 28% speak mostly English. 46% of them read better in Chinese and speak mostly Chinese at home, but another 46% read better in English and speak mostly English at home. Only 38% speak Chinese only with friends, 53% speak only English or mostly English to friends. 30% think in mostly Chinese, 23% think in Chinese and English about the same, but 45% think in mostly English. 54% watch TV mostly in English with 30% do so mostly in Chinese. Over 73% listen to radio mostly in English with none listening to radio in Chinese. 54% identify themselves as Chinese American with 23% identifying with Chinese or American. 38% have mostly Chinese friends and 46% have some non-Chinese friends. 85% either agree or strongly agree with the statement that "I think of myself as being U.S. American." 69% are proud or very proud of their Chinese background. 69% eat mostly Chinese foods. 58% celebrate Chinese holidays most of the time. In conclusion, there are different degrees of acculturation among Chinese immigrants but the effect on their body weight status was not clear due to the limited number of subjects in this study.

Body Weight Perception and Willingness to Adopt Healthy Eating and Activity Behaviors among Kentucky Adults. ERICA COLEMAN*, LINGYU HUANG, CECIL BUTLER, and CHANGZHENG WANG, Human Nutrition Program, Kentucky State University, Frankfort, KY 40601.

Visitors to the 2011 Kentucky State Fair were recruited to fill out a questionnaire before they were given a free analysis of their body composition (body fat %) with a Tanita TBF-521 body composition analyzer. 60% of overweight men considered themselves normal and 77% of obese men considered themselves only overweight. 15% of normal weight women considered themselves overweight, but only 21% of overweight women considered themselves normal and 51% of obese women put themselves in the overweight category. 80% of the participants would choose vegetables or fruits and nuts for snacks but 30% of the obese group would choose chips for a snack. 70% of the participants would learn to prepare vegetable dishes on their own but only 14% would do so by attending free workshops. 72% of participants were willing to add physical activities to their daily life such as walking but only 10% were willing to join a free club for exercise and 10% of the obese is willing to pay for an exercise program. 60% of the obese group was willing to cut soft drinks and 50% of the normal weight and overweight individuals were willing to drink water only. In conclusion, self-perception of body weight tended to lower the severity of weight problems in both men and women. Furthermore, self-perception of body weight status could affect the willingness to adopt healthy eating and activity behaviors.

BOTANY

Effect of Natural Plant (*Cocos nucifera*) Derived Oil on Ulcerative Colitis in a Murine Model. PRANAV CHANDRA*, and NILESH SHARMA, Ogden College of Science and Engineering, Department of Biology (TCNW), Western Kentucky University, Bowling Green, KY 42101.

Ulcerative colitis (UC) is a chronic disease of the colon or large intestine that causes inflammation and ulceration (tiny open sores) of the inner lining of the colon and rectum. Ulcerative colitis can occur in all areas of the colon. In patients with ulcerative colitis, the body's immune system over-reacts and body mistakes food, bacteria, or other internal materials in the colon for an invading substance and it signals the immune system to attack the material, thus irritating the colon. This irritation triggers a flare of ulcerative colitis symptoms likewise bloody, pus or mucus filled stools, diarrhea, cramping, abdominal pain and bloating. Highest incidences are seen in the United States, Canada, the United Kingdom and Scandinavia. Since the etiology of UC remains unclear, successful treatment strategies targeting large sections of affected population have not been found. UC is currently treated with medications that include a combination of anti-inflammatory, immunosuppressive and antibiotic drugs with limited remission and significant episodes of side effects; often patients become refractory and seek an alternative therapy. Lack of efficacious drugs to treat patients with different forms of inflammatory bowel disease underscores need for the development of a new and effective alternative therapy. Currently, the role of saturated fatty acids on human health is being revisited, and this issue is drawing significant attention specifically in inflammatory and metabolic disorders. Effects of medium-chain saturated fatty acids (MCFAs) - like lauric and caprylic acid- have been little studied, and thus drawing much attention. Natural coconut (*Cocos nucifera*) oil is a rich source of MCFA, main constituent being lauric acid: a 12C-chain of fatty acids. Traditionally, coconut oil has been used as cooking oil in several parts of India and other Asian countries. Lauric acid converts to the fatty acid monolaurin in our body and has adverse effects on several microorganisms including bacteria, fungi, yeast and enveloped viruses. Lauric acid is one of the main components of human breast milk, and boosts immune system of children during infancy. Limited knowledge of inflammatory conditions coupled with a narrow range of therapeutic options necessitates investigating the role of natural products. Therefore, the present study focuses on the anti-inflammatory role of natural

fatty acids derived from *Cocos nucifera* in the murine model of ulcerative colitis.

Genetic Diversity in Kentucky Spicebush Populations Using Simple Sequence Repeat Markers. RE'GIE SMITH*, KIRK W. POMPER, JEREMIAH D. LOWE, JACOB BOTKINS, and SHERI B. CRABTREE, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355.

Spicebush (*Lindera benzoin* L.) is an aromatic small native shrub that grows in the moist, understory areas of Appalachia and has potential as a new niche crop for small farmers. Native Americans and early settlers used this plant traditionally as a tea. The berries can be used for jam and spicing of foods, and may have health benefits including antioxidant compounds. Native spicebush patches also can serve an important role in forest ecosystems in terms of fruit production for animals, soil erosion control, and enhancing insect biodiversity. Spicebush may serve to hold ecological niches by outcompeting invasive plants compared to those in unchallenged areas. Genetic diversity of native spicebush populations in Kentucky has not been examined. The objective of this study is to determine the genetic diversity in spicebush populations in Kentucky using simple sequence repeat (SSR) DNA marker systems. Leaf samples were collected from 20 spicebush plants in the forests at the Kentucky State University Environmental Education Center (EEC) and at a location near the Kentucky River. DNA was extracted using the DNAmite Plant Kit. Primers A7, A115, B105, and B122 were used to amplify SSR products that were separated with a 3130 Applied Biosystems capillary electrophoresis system. The software program Power Marker was used to examine genetic relationships among genotypes. The SSR markers generated showed genetic variation among the spicebush genotypes. A number of selections with unique genotypes will be sampled and propagated for study in the KSU germplasm collection for potential cultivar development.

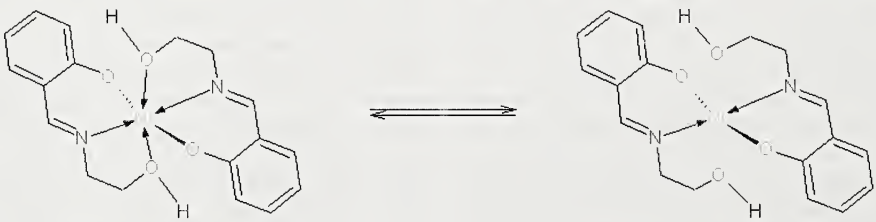
Pawpaw Patch Genetic Diversity and Clonality and its Impact on the Establishment of Invasive Species in the Forest Understory. JACOB BOTKINS*, KIRK W. POMPER, JEREMIAH D. LOWE, and SHERI B. CRABTREE, College of Agriculture, Food Science, and Sustainable Systems, Kentucky State University, Frankfort, KY 40601-2355.

The pawpaw (*Asimina triloba*) is a native understory tree of 25 states of the east and midwest United States. Pawpaw's ability to compete with local invasive species in Kentucky has not been examined. The objectives of this study were two-fold: to determine the genetic diversity and clonality displayed in seven native pawpaw patches located at the Kentucky State University Environmental Education Center (KSU-EEC), the Kentucky River, Cove Spring Park, and the KSU Research and Demonstration Farm in Franklin County, using microsatellite markers; and to determine if patches reduced the incidence of invasive species. Twenty-five trees from seven patches in the four different locations were sampled for genetic analysis. Leaf samples were extracted using the DNAmite Plant Extraction Kit and products from four microsatellite loci were analyzed using a 3130 Applied Biosystems capillary electrophoresis system. String grids were created and invasive plants counted in three 10-meter squares in each of the patches and control plots outside of each patch. The number of plants for each invasive species within pawpaw patches was counted and compared to a control plots. Pawpaw patches displayed high genetic diversity among populations. Japanese honeysuckle (*Lonicera japonica*), garlic mustard (*Alliaria petiolata*), winter creeper (*Euonymus fortunei*), and colts foot (*Tussilago farfara*) were found in most locations; however, there was no significant difference in the incidence of invasive plants between the patches and the control plots. Pawpaw stem density may be important in the incidence of invasive plants within patches.

CHEMISTRY

Synthesis of Homoleptic Nickel (II) Complexes and Examination of their Coordination Dynamics in Solution. LAURA BISHOP*[1], DAVINDER KUMAR[2], CRAIG A. GRAPPERHAUS[2], and CHRISTOPHER S. MULLINS[1], [1]Division of Natural Science, Campbellsville University, Campbellsville, KY 42718, and [2]Department of Chemistry, University of Louisville, Louisville, KY 40292.

In this study, we have begun to examine the structural dynamics for a group of homoleptic nickel (II) complexes. All of the tridentate ligands have one fairly acidic proton attached to an oxygen or nitrogen donor atom that ligates the metal in the first coordination sphere. The ligand 2-(salicylideneamino)-1-hydroxyethane (H_2-SALAHE) has been studied extensively for a variety of applications, including the synthesis of single-molecule



Octahedral structure

Square-planar structure

magnets derived from cluster compounds. A previous publication of the homoleptic Ni(II) complex of this ligand reported the single-crystal X-ray structure, wherein the nickel ion was found to be octahedrally coordinated by two of the ligand molecules. Our recent studies with this complex suggest that the complex undergoes a coordination number change in solution to give a four-coordinate nickel complex. This complex has been found to give green crystals reminiscent of the octahedral structure upon recrystallization. Future work will utilize several spectroscopic techniques such as UV-Visible absorption, EPR, and NMR, etc. in order to study the fluxional nature of these complexes in solution.

COMPUTER AND INFORMATION SCIENCES

Statistical Analysis of Microarray Gene Expression Data from a Mouse Model of Toxoplasmosis. SHRIKANT PAWAR*, CHERYL D. DAVIS, and CLAIRE A. RINEHART, Department of Biology, Bioinformatics and Information Science Center, Western Kentucky University, Bowling Green, KY 42101.

Toxoplasmosis, caused by the protozoan parasite, *Toxoplasma gondii*, is a major cause of morbidity and mortality in patients with AIDS and an important cause of miscarriage, stillbirth and congenital disease in newborns. Previous studies have provided evidence that dietary supplementation with vitamin E and selenium is harmful during experimental toxoplasmosis in mice, whereas a diet deficient in vitamin E and selenium results in decreased numbers of tissue cysts in the brain and dramatically reduced brain pathology. The overall goal of the present study was to determine the impact of dietary supplementation with antioxidants on gene expression in the brains of non-infected mice and in mice infected with *T. gondii* using microarray analysis. RNA was isolated from the brains of C57BL/6 mice, and an Agilent Oligo Whole Mouse Genome Microarray (Agilent Technologies, Inc.) was performed. A total of 48 chips were normalized by Z ratios and the Data Driven Harr Fisch Normalization methods. Differentially expressed genes were identified by applying thresholds to identify significant values and the results were compared between the normalization methods. These differentially expressed genes and their respective fold change ratios were used in Ingenuity Pathway Analysis (IPA) software to analyze the pathways involved with these genes. The identified pathways associated with differentially expressed genes are very important in determining the impact of dietary supplementation with antioxidants on gene expression in the brains of mice infected with *T. gondii* and specific alterations of those pathways can help us in reducing the harmful effects of the same in future. Support from the National Center for Research Resources NIH Grant Number 2 P20 RR-16481 and from the WKU Bioinformatics and Information Science Center is gratefully acknowledged.

ECOLOGY AND ENVIRONMENTAL SCIENCE

Assessing Kentucky State University's Recycling Program. RE'GIE SMITH*, RODNEY RIPBERGER, BRANDAN BURFICT, DUSTIN HODGES, and JOHN D. SEDLACEK, Masters of Environmental Studies Program, Kentucky State University, Frankfort, KY 40601.

The purpose of this study was to conduct a recycling audit of six buildings on Kentucky State University's (KSU) campus and to conduct a survey of the attitudes, perceptions and knowledge of faculty, staff and students about recycling on campus. A six-week audit was conducted of waste from two academic buildings, one administrative building, the student center, and one male and one female dormitory for a total of six campus buildings. Recycling bins were located in the six buildings and their locations were documented on maps. We collected a total of 815 lbs of waste; of the waste that was collected 361 lbs (44%) could have been recycled. Thirty six percent of the recyclables was plastic, 28% was paper and 24% was cardboard. The survey revealed that students recycle less compared to the faculty and the staff. The faculty use the recycling bins an average of 1.32 times per day, staff use the bins an average of 1.47 times per day, and the students use the bins an average of 0.66 times per day. The survey respondents' answers showed that they believe recycling is important and almost 97% are willing to help KSU recycle more. In fact, 81% of the respondents recycled prior to life at KSU, and only 3% consider themselves to not be "green," or to not take actions to promote a healthy environment. The census of students, faculty, and staff provided data that will help make recommendations for the KSU recycling program.

Winter Management of an Invasive Species, Garlic Mustard, *Alliaria petiolata*, in Wooded Habitat. JACOB BOTKINS*, RUSSELL WILLIAMS, ADAM GERUGHTY, and JOHN D. SEDLACEK, Masters of Environmental Studies Program, Kentucky State University, Frankfort, KY 40601.

Garlic mustard, *Alliaria petiolata*, is a biennial cool-season plant growing 0.5–1 m tall. It is an aggressive competitor for resources excluding native plants from their habitats. This plant is shade tolerant allowing it to invade mature woodlands, where it shades out native understory flora and produces allelopathic compounds inhibiting seed germination of other species. It is threatening the federally endangered Braun's rockcress (*Arabis perstellata*) known only from Franklin, Owen and Henry counties. The objective of this research was to quantify two methods of winter management of garlic mustard at the Julian Savanna State Nature Preserve in Franklin County, Kentucky. Hand removal, a 2% glyphosate solution and untreated control treatments were used. Plots were 1 m^2 in area. A thatching rake was used to hand weed while a hand held 0.5-liter sprayer

was used to apply glyphosate to each plot. A digital camera was used to photograph each plot 1.5 m overhead before plot treatment on 16 February. Each plot was photographed nine weeks after treatment and weed control quantified using the NIH ImageJ program (U.S. National Institutes of Health). There was a 22% and 24% increase in garlic mustard and purple deadnettle foliage in glyphosate treated and hand weeded plots, respectively. There was >722% increase in garlic mustard and purple deadnettle coverage in untreated plots. Thus, a single application of glyphosate in mid-February or hand weeding/surface tilling reduces, but does not eliminate, garlic mustard and purple deadnettle in wooded areas.

White Tailed Deer in Frankfort, Kentucky: Population Assessment and Implications for the Community. JON CAMBRON*, TERRELL HOLDER, MARK RASCHE, KIAH RODRIGUEZ, MIKE WARD, and JOHN D. SEDLACEK, Masters of Environmental Studies Program, Kentucky State University, Frankfort, KY 40601.

A spotlighting assessment of the population of white-tailed deer was conducted in five Frankfort city parks. Two to four replications were done on each park. Cove Spring Park's population was estimated at 24, Capitol View Park - 77, Fort Hill Park - 76, East Frankfort Park - 6, and Juniper Hills Park - 0. Based on a calculated estimate of deer/mi^2, Cove Springs and East Frankfort densities fell within the range of expected density based on mean densities of adjacent counties; Capitol View and Fort Hill did not. This could be because surveys of Capitol View and Fort Hill were not accurate or the densities are in fact much higher than expected in this region. To supplement the population survey, we did an informal assessment of the forest understory in three of the surveyed parks and looked at deer-vehicle collisions as a proxy for density. Understory vegetation was limited to a small number of species dominated by bush honeysuckle (*Lonicera* spp.) and very few tree saplings of any species were observed. In Fort Hill Park, the understory was essentially non-existent. There were 851 deer-vehicle collisions between 1 January 2001 and 31 December 2010, mostly occurring in October, November and December. The collision count over ten years, looking at only November, suggests a two or three year deer population cycle. The census combined with the informal vegetation assessment and incidence of deer-vehicle collisions in November suggests that deer in Frankfort may be approaching ecological carrying capacity.

Citizen Awareness of Invasive Plant Species in Kentucky. JOHN D. SEDLACEK*, ADAM GERUGHTY, JACOB BOTKINS, and RUSSELL WILLIAMS, Masters of Environmental Studies Program, and MARA MERLINO, and TIERRA FREEMAN, Psychology Department, Kentucky State University, Frankfort, KY 40601.

Non-native invasive species are one of the primary threats to biodiversity. Public support for invasive species management programs is critical to the success of such projects. Additionally, understanding the public's knowledge, attitudes and perceptions can assist with the development of outreach and educational activities. In order to assess the level of understanding of the invasive plant species threat, attitudes towards invasive species management and demographic factors influencing such attitudes, a questionnaire survey of 400 randomly selected members of the public in the greater Louisville, Lexington, Frankfort and Bowling Green metropolitan service areas was conducted. We developed a survey that determined an elementary level of awareness of invasive plants, people's understanding of what is native vs. non-native, why these concepts matter, and if respondents are motivated to assist in invasive species removal. The survey was administered via Survey Monkey. Surprisingly, only 4.5% of the population invited to participate in the survey actually responded. Thus, drawing major conclusions from the data would not be advisable. However, the low response indicates that the vast majority of citizens are unaware of, or don't care about, the potential economic or ecological consequences of invasive species establishment. We provided all survey information to the Kentucky State Nature Preserves Commission who hopefully will be able to further address educational issues concerning invasive species and how to better market those messages in Kentucky.

GEOLOGY

Nutrient and Fecal Microbe Assessment of the Water Quality of Tates Creek, Madison County, Kentucky. KRISTOPHER H. CARROLL*, and WALTER S. BOROWSKI, Department of Geography and Geology, Eastern Kentucky University, Richmond, KY 40475.

Tates Creek is a significant tributary to the Kentucky River that has shown high levels of microbial and nutrient pollution. We sampled the waters of Tates Creek comprehensively by occupying 25 stations along its 13-mile length, collecting stream water at the confluence of major tributaries from its headwaters to the Kentucky River. Samples were collected four times between May and August 2011 during dry periods as well as immediately after rainfall events. We measured ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^-) concentrations using colorimetry. Microbial samples were measured for total coliform and *Escherichia coli* using IDEXX Colilert-18 media. Background levels of NH_4^+, NO_3^- and PO_4^- are typically ~0.2 ppm, 13 ppm, and 1.0 ppm, respectively. Nutrient concentrations generally increase during rainfall events, presumably because nutrients are flushed into the stream. Background counts of *E. coli* are typically ~100 cfu/mL but microbe counts reached 1000–2419 cfu/mL immediately following rain events. A sewage treatment plant exists approximately two miles from the headwaters and noticeably affects water quality. Nutrient concentration, especially NH_4^+ and PO_4^-, are markedly increased at the plant's outflow. These nutrients then decrease steadily in concentration downstream to background levels. In contrast, fecal

microbe counts are high upstream from the plant, but fall to near-zero levels at its outflow, and then increase anew downstream. The treatment plant went offline on 19 July 2011, so we will be able to assess any changes in water quality and stream health in the future.

Suspended Sediment Concentration in the Brushy Creek Watershed, Kentucky. TYLER A. WADE*, and WALTER S. BOROWSKI, Department of Geography and Geology, Eastern Kentucky University, Richmond, KY 40475.

Suspended sediment concentration (SSC) can be used as a proxy for environmental health of stream water. For example, large sediment loads can cause harm to aquatic life and are a mechanism for introducing and transporting fecal microbes. We measure SSC of the Brushy Creek watershed, located in Rockcastle, Pulaski, and Lincoln Counties, where the Eastern Kentucky Environmental Research Institute (EK-ERI) has been conducting an assessment of the watershed. Two auto-sampling units were placed in Brushy Creek to collect water samples for determination of SSC. The units collect samples every 14 hours for a two-week period, then samples are retrieved for analysis, and new sample bottles are loaded into the auto samplers. Sediment sampling has been in progress since January 2011 and will continue until November 2011. We measure sediment transport during dry, wet, and storm periods. Retrieved samples are brought to the laboratory where sediments are filtered and weighed to determine SSC. The SSC data have been evaluated along with records of rainfall events, as recorded by the UK Agriculture weather station located in Somerset, KY. Due to operational difficulties with our water and sediment samplers, we have only collected intermittent data, however, rainfall events seem to be correlated with increased SSC.

The Micro- and Macro- Faunal Diversity of a Devonian Dysaerobic Environment. LARRY TACKETT*, KARA WELLS, and CHARLES E. MASON, Department of Earth and Space Sciences, Morehead State University, Morehead, KY 40351.

This study examined the fauna contained in the type section of the Three Lick Bed of the Ohio Shale (Upper Devonian), which is located in Rowan County, Kentucky. The Three Lick Bed separates the underlying Huron Member from the overlying Cleveland Member of the Ohio Shale. The unit is 3.42 meters thick and is composed of three greenish gray shale beds separated by two intervening black shale beds. The three greenish-gray shale units were hypothesized to be deposited under dysaerobic conditions and thus the focus of this study. To date slightly over 200 kilograms of samples have been processed for macrofossils and 90 kilograms for microfossils. The samples were broken down using the kerosene technique and washed through a nested set of U. S. standard sieves, a #20 for macrofossils and a #100 for microfossils. The residue caught on the #100 sieve underwent heavy liquid separation and both the heavy and the light fractions were examined for microfossils. All picking, sorting, and identification of fossils were conducted under a binocular microscope. The results of this study support our hypothesis that the greenish gray shale units of the Three Lick Bed were deposited in a dysaerobic environment. Evidence supporting this conclusion includes the following: 1) a low diversity macro invertebrate fauna of 15 species, 2) of the 532 specimens identified nearly all were juveniles, 3) the fauna was dominated by mollusks, 12 out of 15 species, and 4) all macro invertebrates except *Lingula* were preserved as pyretic internal molds. Overall, benthic foraminifera dominate the microfossil fauna in both diversity and abundance, followed by ostracodes in terms of abundance. The macrofossil fauna is dominated by ammonoids being the most diverse (with four species) and a low-spired gastropod being the most abundant (198/532).

HEALTH SCIENCE

Procedure for Preparing Purple Sweet Potato Powder. LINGYU HUANG*, CECIL BUTLER, and CHANGZHENG WANG, Human Nutrition Program, Kentucky State University, Frankfort, KY 40601.

Purple sweet potato has health promoting properties. Purple sweet potato powders currently on the market are made by grinding the raw material and sun-drying the precipitates, or grinding of sun-dried slices of the sweet potato. Recent research indicates that such processes lead to significant loss of antioxidants. Our objective was to develop a process that better preserves the antioxidants when the powders are produced. Purple sweet potatoes were obtained from a North Carolina farm. The whole sweet potatoes were steam-cooked at 200°F for 45 min before they were skinned and mashed. The mashed material was dried in a forced air-drying oven at 60°C or 80°C. After drying the materials were crushed and ground into powder in a Hobart grinder. Cooking of the whole sweet potato avoided the activation of enzymes so the damage of the antioxidants would be reduced. The drying process resulted in 71% loss of weight. The skin accounted for 4.5% of the total weight. The drying temperature was critical. At low temperature, the materials would spoil and mold would grow, rendering the materials useless. At high temperature (100°C), browning of the materials occurred potentially damaging the nutrients. Drying process did not significantly reduce the total phenolic content of the powder.

PHYSIOLOGY AND BIOCHEMISTRY

Regulation of EMT Proteins in Breast Cancer Cell Lines. MARY WIECHART*, JACKIE JANSEN, ARIELLE MARASLIGILLER, HILLARY RESTLE, SHANE MULVIHILL, STEFAN SIWKO, and JULIA CARTER, Wood Hudson Cancer Research Laboratory, Newport, KY 41071.

During 2011, 232,620 new breast cancer cases are predicted in the US and 39,970 breast cancer deaths. This

high mortality rate is due to tumor metastasis. To metastasize, breast cancer cells must undergo epithelial-mesenchymal transition (EMT), a process that disaggregates the epithelium, reshapes it for movement, and requires biochemical re-programming. Slug and p21 activated kinase (Pak 1) are two proteins that are increased during EMT. Another protein, eukaryotic initiation factor 4E (eIF4E), is elevated in breast cancers. Elevated eIF4E function selectively enhances the translation of mRNAs with long, highly structured untranslated regions (UTRs) such as Slug and Pak 1. We hypothesized that elevated eIF4E function in breast cancer cells may enhance translation of Slug and Pak 1, thereby promoting EMT. To test this hypothesis we examined protein expression in western blots of lysates from 6 breast cancer cell lines with different estrogen, progesterone, and Her 2 receptor status. Since MDA 231 breast cancer cells expressed all three proteins, are negative for all three receptors and are reported to be the most invasive breast cancer cell line, we used this cell line to determine if knock down of eIF4E by siRNA transfection would alter expression of these EMT associated proteins. We found a slight reduction in Slug expression in MDA 231 cells with reduced eIF4e but a slight increase in Pak 1 expression. Although these data are preliminary, they do not support our hypothesis that eIF4E regulates Slug and Pak 1 expression and possibly EMT in breast cancer.

Two Germline Variants of the *TGFβR1* Gene are Associated with Initiation, Progression and Clinical Outcome of Colorectal Cancer. HILLARY RESTLE*[1], SHANE MULVIHILL*[1], JONATHAN BENDER[1], KEVIN MURRAY[1], JESSICA SHAW[1], BRIANA VOGT[1], ROBERT SHIELDS[1], BRUCE COLLIGAN[1], JAMES DEDDENS[2], LARRY DOUGLASS[1], JAMES SCHAEPER[1], and JULIA CARTER[1], [1]Wood Hudson Cancer Research Laboratory, Newport, KY 41071, and [2]University of Cincinnati, Cincinnati, OH 45221.

Germline variations of transforming growth factor beta (TGFβ) are associated with tumor initiation and progression, especially in bladder, breast, ovarian, kidney, and lung cancer. Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. There will be over 140,000 people diagnosed with CRC this year and nearly 50,000 CRC related deaths. CRC is frequently diagnosed in the later stages due to the non-specific symptoms in its early stages, further emphasizing the need for genetic biomarkers. Two germline variants in the *TGFβR1* pathway were analyzed via capillary electrophoresis in 233 cases and 219 controls to determine if their incidence affected the prevalence and stage of cancer. We hypothesized that these variants could be significant factors in predicting initiation, progression, and growth in CRC. We found that patients with *Int7G24A*, a single nucleotide polymorphism in the intron 7/exon 7 boundary of the *TGFβR1* gene, had a significantly higher incidence of CRC as compared to non-cancer controls. *TGFβR1*6A*, a nine base pair deletion in exon 1 of the *TGFβR1* gene, was not associated with increased CRC incidence but was associated with adenoma patients that did not progress to advanced CRC. This suggests that *TGFβR1*6A* may have a protective effect. Patients with carcinoma-in-situ (CIS) or CRC stages 1–4 showed a significantly increased incidence of the *Int7G24A* variant as compared to non-cancer controls and patients who never progressed beyond adenoma. This discovery indicates that *Int7G24A* could be a biomarker for identifying patients at a higher risk for developing CRC.

SCIENCE EDUCATION

Sharing Ideas About Assessing Student Learning. JOHN G. SHIBER, Division of Nursing, Biology & Allied Health, Big Sandy Community & Technical College, Prestonsburg, KY 41653.

An emerging national policy of holding post-secondary educators more strictly accountable for student learning has many scrambling to re-evaluate their teaching strategies and the assessment parameters they employ. It is an overwhelming challenge because, as colleges increasingly become like businesses and treat their students like clients, student attitudes toward learning are undermined by an equally strong if not stronger one of entitlement, irrespective of how much or little they apply themselves in their studies. This paper discusses the consequent need for an increased number of parameters, besides testing, to help assess student learning in the sciences such as those shown in studies by this investigator to be beneficial: pre-/post-testing, class attendance, in-class writing assignments immediately after reading articles or watching videos on scientific topics, active individual involvement and course-appropriate extra credit opportunities in and beyond the classroom, end-of-semester student opinion questionnaire on course, etc. An argument for establishing continuity among teachers within each science discipline to follow the same assessment guidelines will also be presented.

ZOOLOGY

Measurement of Differential Acid Concentration Along the Developing Gastrointstinal Tract of Tadpoles with an Improved pH Microprobe. SARAH E. CROSS, and RICHARD D. DURTSCHE, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

Digestion and assimiliation of foodstuffs in vertebrates is often dependent upon changes in the chemical environment along the gut depending on the level of food decomposition and the optimality of conditions for enzyme activity. Low pH in the stomach can chemically breakdown food while activating pepsin protein enzymes. Other areas of the gut (e.g., colon) could show lower pH levels that would indicate plant fermentation and the release of volatile, short-chain, fatty acids. Measurements across developmental stages can document ontogenetic shifts in acid concentrations in the gut suggesting

upregulation of digestive activites within the gastrointestinal (GI) tract. As free living vertebrates, tadpoles (anuran larvae) undergo developmental changes in the formation of the GI tract, but they are also consuming foods as might an adult vertebrate. Functional changes in digestive processes in tadpoles can therefore be responses to either maturation of the system or a response to the varied foods consumed. Previous research in our lab has shown changes in pH across the GI tract, suggesting digestive processes similar to adult vertebrates. Our continued investigations of differential acid concentrations along the gut has resulted in the design and fabrication of an improved pH microelectrode with a built-in micro reference electrode. We have been testing this new microprobe on locally collected Green frog (*Lithobates clamitans*) tadpoles, where after dissection, various regions of the GI tract were measured for changes in pH. In testing our solid-state microelectrodes with tip diameters of =10 μm against standard pH solutions, we maintained precision of $R^2 = 0.95$.

Beyond mtDNA: Morphology and Nuclear Gene Flow Suggest Taxonomic Oversplitting in the Ringneck Snake *Diadphis punctatus*, FRANK M. FONTANELLA, Department of Biology and Chemistry, Morehead State University, Morehead, KY 40351.

Being able to efficiently and accurately delimit species is one of the most basic and important aspects of biology because species are the fundamental unit of analysis in biogeography, ecology, and conservation. This delimitation may be hampered by variation within and between populations making it difficult to determine whether populations have evolved into independent evolutionary units. Recently there has been a resurgence in species delimitation beyond traditional morphological and mitochondrial data that incorporates species distribution modeling and nuclear data to assess ecological divergence and levels of gene flow between populations. Using the Northeastern ringneck snake *Diadophis punctatus edwardsii* as a model, I expanded upon previous work by combining 24 external meristic characters from 300 museum samples, species distribution models generated from 19 climatic variables derived from 415 unique locality data points, and 10 microsatellite loci from 288 individuals, to test whether the mtDNA clades represent distinct evolutionary units. The PCA and CVA analysis of the meristic data failed to recover significant differences between the two mtDNA clades. Demographic analysis of the mtDNA data depicts rapid population expansion of the northern clade that corresponds to large areas of shared suitable habitat predicted by the species distribution models. Likewise, admixture analysis of the microsatellite data suggests high levels of nuclear gene flow between populations. When combined, these results suggest that the mtDNA clades are likely the result of historical divergence followed by contemporary gene flow. Moreover, this study highlights the importance of incorporating multiple lines of evidence for populations suspected of being cryptic species.

A Dissolved Oxygen Microprobe for Measuring Gut Anaerobic Fermentation in Developing Vertebrates. KATHERINE BACHMAN, KELSEY CARNAHAN, and RICHARD D. DURTSCHE, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

Food assimilation in organisms is important in the extraction of energy. Previous research by both our lab and others suggest anurans are herbivorous and detritivorous feeders. The extent to which cellulose breakdown and fermentation occurs in the GI tract is unknown. Studies done on pH in the GI tract of *Lithobates clamitans* has shown two major drops in pH, in the stomach and hindgut. The use of a dissolved oxygen microelectrode to measure oxygen levels in the gut would give us an idea of possible locations of cellulose fermentation and the possible existence of a colon. Over the past year, our lab has been working on building a dissolved oxygen microelectrode probe to measure these levels. Assembly of these probes is comprised of four main components: the cathode, anode, electrolyte solution and outer casing. The cathode is a glass fused solid-state capillary tube, with a gold tip. The anode is silver wire coated in chloride ions. The outer casing is a glass pipette with a tip diameter of 10 micrometers and gas permeable membrane. The electrolyte is a mixed potassium chloride solution maintained at a high pH. These probes were calibrated with a high degree of accuracy and precision with rapid response times using standard concentrations of dissolved oxygen in water. Regression analyses against standard solutions have R^2 values in the range of 95%. Gastrointestinal tract samples were obtained from *Lithobates clamitans* and readings taken from 15 positions along the gut.

Amphibian Population Dynamics of a Rejuvenating Brown Field. JAMES 'MITCH' MERCER, and RICHARD D. DURTSCHE, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

The Lafarge Gypsum Plant, located in Silver Grove, KY, includes both secondary growth forest consisting of varied hardwood species and open wetland habitats. Just south of the Ohio River, grassland between the secondary forest and the riverbank supports vernal ponds, inundated with water during the spring. Approximately 5 cm of top soil exists due to the land that once served as a railroad yard, the underlying soil being permeated with rock. Determined a brown field during this stage development, chemicals due to industrial waste may remain on site despite rejuvenation efforts. Amphibians are especially susceptible to these environmental conditions as their moist skin facilitates a plethora of life processes. The study was conducted to analyze the ability of the site to sustain wildlife post-rejuvenation efforts. A comparative non-impacted wetland site (St. Anne Wetlands) approximately 2 km away was added to compare species thriving at either location. Various field techniques led to the capture of several species approximately every other day for a span of

two years (during months of activity) to understand the dynamics of populations at either site. Species were marked to track migration between sites. Field sound recorders were implemented to track potentially unobserved species. Results suggest that the Lafarge site is able to sustain some reptile and amphibian species, however, several species, in particular salamanders, thrive at the St. Anne wetlands but have not settled at Lafarge.

JUNIOR ACADEMY OF SCIENCE

ENGINEERING

Lubrication Efficiency of Oil Weights in Engines. GABRIEL L. M. WEBB-YEATES, Bowling Green High School, Bowling Green, KY 42101.

Engine oils have different weights such as 5W-30 and 10W-30. Each oil weight has a different viscosity and lubrication ability. High weight oil is more viscous. Clean oil should be less viscous than used oil. My hypothesis is that lower weight oils will lubricate better at cold temperatures. Clean oil should lubricate better than dirty oil. The relative viscosity of different weights of oil from the same manufacturer increased for heavier weights. For the same weight of oil from different manufacturers, the relative viscosity is similar. Used engine oil was more viscous. Lubrication ability was measured by putting a fixed volume of oil between two metal plates placed on a self-manufactured adjustable inclined plane. The height of the inclined plane when the top metal plate starts to slide or slip over the bottom plate was used to measure relative lubrication. Engine oil of the same weight from different companies was measured. Different weights of oils from the same company were measured. Both clean and used oil of the same weight were measured to see if lubrication changes as the oil becomes dirty and used. The inclined plane worked well with good consistency in the slip point height. For the same manufacturer, the lightweight 5W-30 oil lubricated better at cold temperatures than heavy weight oils. Clean oil lubricated better than dirty oil. Surprisingly, high mileage engine oil, which will stick to metal parts better, had a higher slip point than other oils of the same weight.

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Erratum: David D. Taylor was omitted as one of the contributors to *Micropropagation, Cryopreservation, and Outplanting of the Cumberland Sandwort Minuartia cumberlandensis* from the Fall 2011 issue of the Kentucky Academy of Science.

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